The Chromosomes

M. J. D. WHITE

Professor of Zoology in the University of Melbourne

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CHAPTER I

The Interphase Nucleus

We shall be concerned in this book with the physical mechanism of nuclear inheritance in the higher organisms. In the bacteria masses of material chemically similar to the chromosomes of animal and plant cells exist, but we do not have as yet any certain picture of the precise organization of the hereditary material in these forms, in spite of much precise, elegant and brilliantly successful genetic work on various species of bacteria. Bacterial cytology has simply not reached the stage where it can be correlated with that of higher organisms with any assurance (somewhat overconfident claims to the contrary notwithstanding). It is nevertheless clear that the genetic material of bacteria and viruses is organized in a linear manner, i.e. that it exists as bodies which we may call chromosomes, although their behaviour in replication, cell-division and genetic recombination may differ considerably from the mechanisms present in higher organisms.

On the other hand, the nuclei and chromosomes of animals and plants, including the Protozoa, Protophyta and Fungi, do seem to possess a fundamental unity of structure and behaviour in spite of great diversity of detail. This suggests that what we may call the fully evolved chromosomal mechanism, including the fundamental processes of mitosis, meiosis and fertilization, arose in an essentially monophyletic manner early in Precambrian times, probably over 2×10^9 years ago.

The 'fully evolved chromosomal mechanism', which eventually made complex multicellular life possible on earth, must have arisen from the quite complex but apparently very different kind of genetic mechanism found in the bacteria. Perhaps we might call the genetic mechanisms of bacteria prochromosomal, those of the Protista and all higher organisms, metachromosomal. If so, this book is about metachromosomal mechanisms. But it is hardly possible as yet to draw up a clear unambiguous list of differences between prochromosomal and metachromosomal systems. It is unlikely that any still existing species of Protista exhibit intermediate evolutionary stages between prochromosomal and metachromosomal mechanisms and various aberrant types of mitosis and meiosis which have been described in some Protista more probably represent specialized modifications of the normal metachromosomal cycle.

Interphase, interkinetic or 'resting' nuclei are ones which are not undergoing the visible changes involved in mitosis. From a physiological standpoint we may perhaps distinguish between interphase nuclei which are between two mitotic cycles and those which seem to have entirely lost the capacity to undergo mitosis. Early embryonic tissues contain only the former, while adult tissues may be composed entirely of one type or the other or perhaps, in some cases, of a mixture of both kinds.

Any general description of the appearance of interphase nuclei is rendered difficult by the fact that a bewildering number of different kinds exist. This is especially so in the higher animals such as insects or vertebrates, with great histological complexity. Plants are, in general, histologically much simpler and accordingly show less variety of interkinetic nuclei.

We may distinguish between those features of the interkinetic nucleus which are characteristic of the

species and those which are associated with particular tissues. Certain species or higher taxonomic entities such as families or orders show relatively large or small nuclei in all or almost all their tissues. Thus in the Urodeles nuclei are always large, i.e. no really small nuclei exist, while in birds most tissues contain small nuclei. On the other hand, certain tissues show the same type of nuclei over a wide taxonomic range of organisms.

Interkinetic nuclei usually remain optically unaltered for long periods of time – they are not obviously changing either their shape or appearance. But this is not always so; many interkinetic nuclei grow in size and may change in shape or appearance in various ways without dividing.

The chief structural elements of the interkinetic nucleus are the *nuclear membrane*, the *nuclear sap* and the *chromosomes*. *Nucleoli*, frequently conspicuous in interkinetic nuclei, are in reality specialized parts of the chromosomes although their structure is such that from certain standpoints we must almost consider them as a distinct kind of nuclear constituent.

The nuclear membrane has been studied by microdissection and in electron micrographs. Both techniques show that it is a definite structure with considerable strength and thickness. If a fine needle is pushed against it, it becomes indented at the point where the pressure is applied. If the pressure is released it regains its former shape while if the pressure is increased it can be punctured (once perforated, the nuclear membrane is apparently unable to repair the injury and the nucleus is killed). Electron micrographs have shown that the nuclear membrane of the amphibian oocyte consists of two distinct layers, an outer sieve-like one about 300 Å in thickness, with numerous 'pores' 400 Å in diameter; and a thinner inner layer without pores. The outer

porous layer seems to contain both protein and lipoid molecules and is mechanically weak, while the thin, tough inner layer is probably pure protein. Different types of nuclear membranes have been described in other kinds of animals and plants, but in all cases the structure is relatively complex. The nuclear membrane is positively charged, while the chromosomes usually seem to carry a weak negative charge.

Most nuclei are approximately spherical, but many are ovoid. Those of many vertebrate leucocytes are in the form of a long strand with periodic enlargements, and the same kind of shape is exhibited by the macronuclei of some ciliate Protozoa such as *Condylostoma* and *Ephelota*. Certain types of secretory cells in insects (silk gland cells of caterpillars, salivary gland cells of pond-skaters) have irregularly branched nuclei. In all these instances of non-spherical nuclei the nuclear surface is large relative to its volume and it has been suggested that this surface/volume relationship is functionally related to the secretion process; but many secretory cells, including some in which the nucleus is very large (such as the salivary gland cells of Dipterous larvae), have spherical nuclei. It is perhaps significant that most types of nuclei with extravagant shapes are highly polyploid (i.e. they contain many chromosome sets, so that there may be thousands of chromosomes in each such nucleus). But many highly polyploid nuclei have a simple spherical or ovoid shape.

In a few instances the interkinetic and prophase nucleus is a vesicular structure, each chromosome being enclosed in a separate membrane or 'karyomere'. The classic example of this state of affairs is in the early cleavage nuclei of the mite *Pediculopsis*.

In living cells, the interphase nuclei may appear 'optically empty' when seen in bright field or dark field illumination, but this is apparently only because the

differences in refractive index between the chromosomes and the nuclear sap are relatively slight. With phase contrast optical systems a considerable amount of structure can be seen in living nuclei. Most chromosomes apparently contain numerous short segments which remain condensed during interphase. These are the so-called heterochromatic blocks or granules, which impart to the fixed and stained nucleus its finely or coarsely granular appearance. They can also be seen in living nuclei by phase contrast. The actual chromonemata or chromosome threads can almost certainly be seen in some types of interphase nuclei, by phase contrast. In many types of nuclei several or many large blocks of condensed material are conspicuous throughout interphase as the so-called *prochromosomes* or *chromocentres*, which represent large heterochromatic segments in some of the chromosomes – i.e. regions that do not undergo de-condensation at the end of mitosis but remain in a state that resembles the metaphase condition throughout the interkinetic period. In certain types of somatic nuclei, especially in insects, all the chromosomes may remain in a semi-condensed condition during interphase, so that although their outlines are diffuse and 'fuzzy', they can nevertheless be counted. Quite a different type of interkinetic nucleus is formed in certain tissues – chromocentral masses are present, but they all aggregate to one side of the nucleus so as to form a kind of cap, the rest of the nucleus appearing relatively empty, in a stained preparation (actually, it undoubtedly contains the non-heterochromatic segments of the chromosomes, in a diffuse condition).

The apparent reticulum or network seen in many fixed and stained nuclei is certainly to some extent an artifact, due to the irregular collapse and anastomosis of many of the chromonemata under the influence of the fixing agent.

In many species of animals and plants one or more nucleoli are very conspicuous structures during interphase, but in others no distinct nucleoli can be seen. When we come to consider the mitotic chromosomes we shall have more to say about these structures. They are always attached to particular chromosomes.

Certain types of nuclei contain masses of material floating around in the nuclear sap which are probably products of chromosomal activity and may be extruded into the cytoplasm through the nuclear membrane. Such materials have frequently been confused with true nucleoli, to which they may be similar in chemical composition.

The visible differences between the interkinetic nuclei of the various tissues of a species may be regarded as an expression of cellular differentiation. We shall discuss this question again in connexion with the giant polytene nuclei of the Diptera (Ch. IV), but it can be stated now that at least some differences between the nuclei of different tissues seem to arise as a result of the swelling up of particular chromosomal segments in one type of tissue and not in another. If this viewpoint is correct, they are a rather direct result of genic activity.

The nature of the factors which induce the onset of mitosis in cells which are 'competent' to undergo division is not well understood. In several vertebrate tissues it has been shown that inherent 'mitotic rhythms' exist, related to periods of activity and rest or to the alternation of night and day. Such rhythms may be related to the blood glucose level. In plant root tip meristems there may be periodic waves of mitotic activity which persist even at constant temperature and in complete darkness.

Where a number of nuclei are enclosed in a common mass of cytoplasm they always seem to undergo mitosis simultaneously and keep in step, so that they are all in

exactly the same stage of division at any instant. Thus in the yolky eggs of insects the first eight or nine cleavage divisions usually take place without the formation of any cell-walls between the nuclei, which go on dividing in a strictly synchronous manner until there are about 512 of them in the embryo, when cell membranes are formed. As soon as this happens the division cycles of the nuclei begin to get out of step, and some may be in various stages of mitosis while others are in the resting stage. Presumably the substances initiating mitosis or controlling its rate can diffuse freely through the yolky cytoplasm but are unable to pass through the cell membranes.

Synchronous division is not confined, however, to such syncytia or multinucleate cells. In the testes of insects the spermatogonia and spermatocytes are enclosed in cysts or packets of 2, 4, 8, 16, 32 . . . cells and the divisions are, as a rule, strictly synchronous within each cyst, in spite of the cell membranes between the nuclei. It is possible that in this case there are cytoplasmic 'bridges' between the cells and that substances controlling the divisions can pass from cell to cell through these 'bridges'.

Chromosome chemistry

Chromosomes are composed of two kinds of nucleic acids and two main types of proteins. A great deal is now known about the chemical constitution of each of these; but we are still largely in the dark as to just how they are combined to form the visible body of the chromosome. A small amount of calcium also seems to be present in chromosomes and may play an important role in joining together successive segments. Suggestive evidence for this view comes from the fact that chelating substances such as versene which bind calcium cause disintegration of chromosomes into minute fragments.

Traces of lipid materials may also be present in chromosomes, at least in some instances.

The nucleic acids are fibre-like molecules whose length is much greater than their diameter. They are polymers of nucleotides, units whose molecular weight is somewhat over 300. The main nucleic acid of the chromosomes is desoxyribonucleic acid (DNA). The other kind of nucleic acid in the chromosomes is ribonucleic acid (RNA) which also exists in the cytoplasm. Each of the nucleotides which are the building blocks of DNA consists of a molecule of phosphoric acid, a pentose sugar (d-2-desoxyribose) and a basic group which may be either a purine (adenine or guanine) or a pyrimidine (thymine or cytosine). In certain organisms a few of the nucleotides may contain 5-methyl cytosine. In the polymerized molecule the sugar and phosphate groups alternate to form the backbone. Apparently the number of adenine bases is always the same as the number of thymine groups and, similarly, the number of cytosine and guanine bases is always the same for any particular sample of DNA. However, DNA's from different species vary widely in the adenine/guanine and thymine/cytosine ratios which appear to range from 0·7 to 1·7. DNA samples from different tissues of the same species are all identical in composition.

According to the now generally accepted interpretation of WATSON and CRICK the polymerized DNA molecule is a double-stranded affair wound into a helix. The two strands are complementary so that wherever an adenine base occurs in one a thymine base is present at the corresponding level in the other and similarly for guanine and cytosine. The two strands are held together by hydrogen bonding between the bases. The total length of the DNA in a single chromosome amounts to several centimetres, but it is uncertain whether it exists in the chromosome as a single fibre; numerous shorter

lengths with protein segments in between is an alternative possible structure.

However it is distributed in the visible chromosome, there can be little doubt that the DNA of the chromosomes is the carrier of all the genetic specificity. We may liken it to a long tape imprinted with words in the morse code. Just as the morse alphabet depends only on two elementary symbols (dot and dash), so the DNA code consists of only four main symbols – the nucleotide pairs A-T, T-A, C-G and G-C (the role of 5-methyl cytosine is obscure at present). In the haploid chromosome set of the human species there are altogether about 4,000,000,000 of these nucleotide pairs.

Earlier statements that the oocyte nuclei of some invertebrates contain no measurable amount of DNA were probably based on inadequate technique. Certain viruses such as tobacco mosaic virus and influenza virus do certainly lack DNA, but contain RNA instead – it is thus probable that in them genetic specificity is carried by RNA or ribonucleoprotein. Bacteriophages contain DNA but no RNA. The fact that highly purified DNA preparations cause specific genetic changes in bacteria (the 'transformation' phenomenon) is a rigorous proof that this substance can carry genetic 'information'.

Ribonucleic acid (RNA) differs from DNA in that the sugar is ribose instead of desoxyribose and the pyrimidine uracil replaces the thymine of the DNA molecule. The amount of RNA in the chromosomes seems to vary from tissue to tissue; thus in calf thymus chromosomes it is only one-fortieth as abundant as DNA, while in liver chromosomes it is one-tenth as abundant. RNA is also found in large quantities in the cytoplasmic particles. There is no certainty that it is polymerized to the same extent as the DNA. In the chromosomes it is probably combined with histones

but the precise mode of combination is not understood. The nucleolus in some cases contains large quantities of RNA. It is now generally believed that RNA plays an essential role in protein synthesis and that the chromosomal RNA, at any rate, may be formed alongside DNA, having a specific sequence of nucleotides built into its structure by a 'template mechanism'.

The chromosomal proteins consist of (*a*) basic histones and protamines, compounds of about 10,000 molecular weight in which the diamino acids arginine and lysine predominate, (*b*) 'residual' protein containing considerable quantities of tryptophane and having a much higher molecular weight. Histone and protamine appear to be equivalent alternatives in the structure of the chromosome; thus in the Salmon the erythrocyte and spermatogonial chromosomes contain histone, but in the ripe sperms protamine is present instead.

Studies of the nucleic acid composition of the chromosomes have been facilitated by the use of highly purified enzymes which are specific for the breakdown of DNA and RNA. Similarly, it has been possible to make use of the fact that histones are soluble in sulphuric acid, whereas the tryptophane containing protein is not, to determine the relative amounts of the two types of compounds.

The synthesis of new DNA takes place during interphase, and in fact during a limited part of the interphase; in root tip cells of *Vicia faba* it extends over a period of about 8 hours and is completed 8 hours before the next metaphase.

The relationship between the synthesis of DNA and the formation of chromatids which will later separate at anaphase as daughter chromosomes has been studied by means of radioisotope techniques. TAYLOR has incorporated thymidine labelled with tritium (H^3) into chromosomes of root tip cells. Because of the very low

turnover rate of DNA, once labelled it stays labelled. The results of the experiment are studied in autoradiographs, the low energy of the β-particles from tritium ensuring high resolution. The drug colchicine was used in this experiment to retain the two sets of daughter chromosomes within the same nucleus.

The result and interpretation of the experiment is shown in Fig. 1. Apparently the chromatid is two-stranded even in early interphase. When DNA synthesis occurs two new strands are formed. If 'tritiated' DNA is present these new strands will be radioactively labelled. In the following mitosis each chromatid will consist of an old (unlabelled) strand and a new (labelled) one. Thus in the autoradiograph both daughter chromosomes at anaphase will appear equally labelled (in reality half labelled). If such cells are allowed to go through a second interphase without a supply of 'tritiated' thymidine chromosomes will be produced consisting of an unlabelled and a labelled old strand and two unlabelled new strands. Thus at the second metaphase following labelling the result is different – only one of each pair of daughter chromosomes is radioactive.

There are some complications in the interpretation of this experiment, but the explanation given by TAYLOR seems likely to survive the criticisms levelled against it. Sometimes a process of somatic crossing-over between sister strands complicates the situation. But the essential fact that seems to emerge from this work is that each chromatid as seen at prophase or metaphase consists, as far as its DNA is concerned, of two 'strands', one 'old' and one synthesized during the previous interphase. Each 'strand' may of course be multiple; it could be a longitudinal half of a DNA molecule – but there are some reasons for thinking that it cannot be a whole DNA molecule, including both longitudinal halves.

The chemical mechanism involved in the duplication of the DNA molecule has been further investigated by

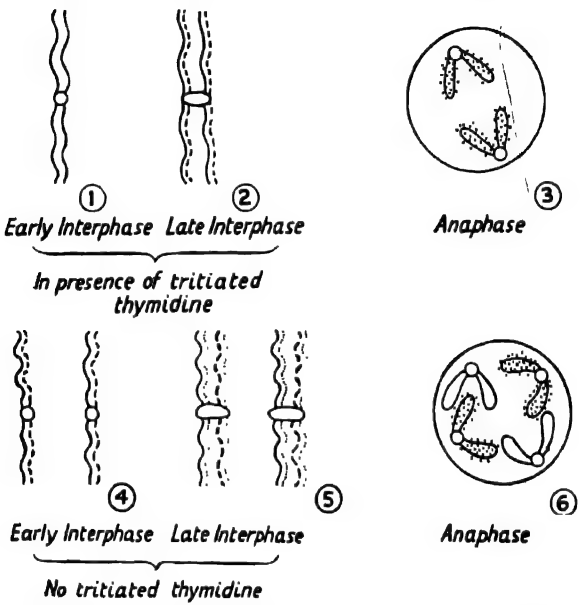

Fig. 1. Diagram illustrating the experiment of Taylor, Woods and Hughes

The first replication takes place in the presence of tritiated thymidine, the second in its absence. Colchicine is used to keep all the daughter chromosomes together in the same nucleus. The original strands are shown as solid lines, the strands formed in the first replication as dashed lines, and those formed in the second replication as dotted lines.

MESELSON and STAHL, by labelling with N^{15} ('heavy nitrogen'), which imparts an increased density to the

molecule. Density gradient centrifugation in a concentrated solution of caesium chloride is then used to separate the labelled and unlabelled macromolecules. 'Half-labelled' molecules one cell generation after changing from N^{15} medium to N^{14} medium can be dissociated by heat (100° C.) into N^{14} and N^{15} containing subunits of half the original molecular weight. This experiment at the strictly molecular level parallels TAYLOR'S experiment at the chromosome level.

We shall speak of the amount of DNA present in a diploid interkinetic nucleus prior to replication as the 'diploid quantity'. There is a good deal of evidence that (with certain exceptions to be noted later) this is a fixed amount which is characteristic of each species of organism and does not vary from one tissue to another. The total amount of DNA in a nucleus may be determined by bulk chemical methods, in which the total amount of DNA in a chemical preparation is divided by the estimated number of nuclei present. Alternatively, it may be measured spectrophotometrically on single nuclei that have been stained by the Feulgen method, which is specific for DNA, and does not stain RNA. In certain tissues, such as mammalian liver, there are always a certain percentage of polyploid nuclei, which contain twice or four times the standard diploid amount of DNA, so that in such cases the bulk chemical method would be inappropriate. The bulk chemical method is also unsuitable for tissues which contain a considerable number of dividing cells, since diploid nuclei contain the 'tetraploid' amount of DNA after replication has taken place until the following anaphase.

Some examples of the 'DNA values' of particular species are given in Table I. It will be noted that wherever it has been possible to measure the DNA content of sperm nuclei, this is approximately half that of the diploid nuclei, of the same species. There is a general

TABLE I

DNA content of nuclei of various species

(in picograms*)

ORGANISM	*Somatic nuclei* (presumed diploid)	*Egg*	*Sperm*
Yeast	0·0062		
Clostridium welchii ('unit cell')	0·0245		
Jellyfish, *Cassiopeia*	—	—	0·33
Sea urchin, *Arbacia aequituberculata*	—	220†	0·67
,, ,, , *Lytechinus*	—	—	0·90
,, ,, , *Paracentrotus lividus*	—	24–26†	0·70–1·1‡
Drosophila melanogaster	0·17	—	—
Limpet, *Fissurella*	—	—	0·50
Squid	—	—	4·50
Lamprey	5·0	—	—
Shark, *Carcharias obscurus*	5·46	—	—
Sturgeon, *Acipenser sturio*	3·2	—	—
Shad, *Alosa*	1·97	—	—
Carp, *Cyprinus carpio*	3·49	—	1·64
Shad, *Eucinostomus guba*	0·94	—	—
Brown trout	5·79	—	2·67
African lungfish, *Protopterus*	100	—	—
Amphiuma	168	—	—
Necturus	48·4	—	—
Frog	15·0	—	—
Toad	7·33	—	3·70
Turtles (several species)	4·92–5·27	—	—
Snakes (several species)	2·85–5·02	—	—
Chicken, *Gallus domesticus*	2·2–2·6‡	—	1·26
Birds (seven species)	1·7–2·92	—	—
Kangaroo, *Macropus rufus*	3·1	—	—
Bandicoot, *Perameles nasuta*	9·2	—	—
Ox	6·0–7·5‡	—	—
Sheep	5·7	—	—
Pig	5·1–6·8	—	—
Horse	5·8	—	—
Rabbit	5·3	—	—

TABLE I (*contd.*)

	Somatic nuclei (presumed diploid)	*Egg*	*Sperm*
Guinea pig	5·9	—	—
Rat	5·7	—	—
Mouse	5·0	—	—
Dog	5·3	—	—
Man	6·0–6·8‡	—	—

* 1 picogram = 10^{-12} gram.
† presumably includes cytoplasmic DNA.
‡ several determinations by different workers, not necessarily using the same method.

tendency for the DNA values to increase from sponges up to vertebrates; but the really outstanding thing about these figures is the extraordinarily high DNA values for certain Urodeles and the lungfish, *Protopterus*. Frogs and toads show lower values, but still much higher ones than the mammals. DNA values acquire a theoretical importance if we regard them as an expression of the total number of genetic loci present. It is difficult, however, to think of lungfishes having twenty times as many genetic loci as teleosts and fifteen times as many as some mammals. Two possible explanations of the high values met with in the Dipnoi and Urodeles would seem to be (1) that these animals have many-stranded (polytene) somatic chromosomes or (2) that they have large blocks of genetically inert or quasi-inert 'heterochromatin' (no definite evidence for either of these hypotheses seems to exist). Species and groups with high DNA values show large somatic nuclei while those with low values exhibit small nuclei, in general. The general evolutionary stability of the DNA values deserves to be pointed out. It seems highly unlikely, for example, that any species of Urodele has a really low value or any bird a high one.

Mass chemical analysis of nuclei or chromosomes

requires their isolation in a reasonably pure state. Within the past 15 years techniques have been developed for preparing suspensions of isolated chromosomes from various types of nuclei such as those of mammalian thymus lymphocytes, liver and kidney cells, the erythrocytes of amphibia and fishes, etc. These methods involve the disintegration of the tissue and breaking of the nuclear membranes by means of a Waring blender or a colloid mill. In some cases quartz sand or diatomaceous earth may be added as a grinding agent. The material is then strained through layers of fine gauze, washed with saline and centrifuged. The chromosomes form a sediment which can be cleaned and freed from debris by further washing with saline. All these operations must be carried out at temperatures only slightly above freezing.

Since chromosomes obtained in this way are derived from resting nuclei, it is somewhat surprising that when examined under the microscope they resemble the prophase chromosomes of ordinary fixed preparations. It is, in fact, rather uncertain what physical changes these chromosomes may have undergone during the procedures of grinding, washing and centrifugation. That they actually are chromosomes is shown by the fact that their appearance corresponds to that of the normal mitotic chromosomes of the species from which they were derived; where the latter are mostly or exclusively metacentric (two-armed), the isolated chromosomes can likewise be seen to be two-armed. In many instances these isolated chromosomes can be seen to be longitudinally split. It has been claimed that some isolated chromosomes have nucleoli still attached to them, but other cytologists were unable to detect nucleoli in preparations obtained by these methods.

There is no particular difficulty in obtaining large quantities of isolated chromosomes, if one simply starts

with enough tissue, and they have naturally been used for studies of their chemical composition.

In various species of animals it has been found that the DNA content of the egg is greater than that of the sperm. What probably happens in such cases is that the egg cytoplasm contains degradation products of DNA, derived from the nuclei of degenerating 'nurse cells' that have been absorbed into the ovum. These materials (possibly in the form of partially or wholly depolymerized DNA) may be utilized in the repeated and rapid synthesis of new chromosomes during the early cleavage divisions. Eventually, at about the gastrula stage in the sea urchin, this store of cytoplasmic DNA or DNA-precursor molecules becomes used up, and thereafter DNA constancy per cell is maintained.

A variety of suggestions have been made as to the relationship between the DNA and protein components of the chromosomes, but no firm conclusions can be drawn. Some workers favour a model in which DNA molecules are attached as side-branches to one or more protein axes. Others prefer a structure in which the DNA molecules are placed end-to-end, possibly with protein internodes. There is still a distinct possibility that each chromatid contains only a *single* DNA molecule, several centimetres in length and complexly folded or coiled. Replication of the DNA molecule would be expected to produce two helices interlocked at each gyre (in the same manner that two parallel lengths of wire will be found to be interlocked if they are spirally wound around a piece of rod which is then withdrawn). Various suggestions have been made to explain the fact that the daughter DNA molecules do undoubtedly manage to separate from one another, but none of these hypotheses has received a conclusive experimental confirmation.

The nucleus and the cytoplasm exist in a state of

symbiosis. The nucleus depends on the cytoplasm for its energy supply and (ultimately) for the materials out of which new nucleic acid molecules and chromosomal proteins can be synthesized. But the cytoplasm clearly depends on the nucleus for the maintenance of some at least of its essential biosynthetic mechanisms. This is, no doubt, the reason why the mammalian erythrocytes, which have lost their nuclei, only have a life of a few weeks. There are some indications that when nuclei are transplanted into the cytoplasm of a different species replication of the genetic material may not proceed normally, presumably because the 'template' chromosome is supplied by the cytoplasm with the wrong building blocks.

CHAPTER II

The Mechanism of Mitosis

The process of mitosis has now been studied in many thousands of different species of animals and plants. Although there are some important differences of detail in the way the cells of different organisms divide, it is remarkable how similar the main stages of cell-division are in plants and animals, from the unicellular algae and Protozoa up to the flowering plants and vertebrates. On the other hand, owing to various technical difficulties in handling the material, it is by no means certain as yet whether the processes of division in the bacteria are similar to those occurring in higher organisms, one school of cytologists maintaining that true mitoses can be recognized in many bacteria, while others emphatically deny that this is so (the controversy will probably be decided, one way or the other, within the next few years).

It is usual to divide mitosis into four stages, *prophase*, *metaphase*, *anaphase* and *telophase*. In some cases it is convenient to designate the transition from prophase to metaphase by the name *premetaphase*. However, the whole cycle is a continuous one and the decision, for example, to call a particular stage late anaphase rather than early telophase may be a fairly arbitrary one. The time taken to complete the whole process, as well as the relative duration of the separate stages, varies greatly in different types of cells, and is also affected by temperature and other environmental factors. The rapid

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divisions that occur in some insect eggs and in certain tissue cultures take a few minutes to half an hour to complete. In grasshopper neuroblasts the entire mitotic process occupies 8 hours at 26° C. and about 3·5 hours at 38° C. The interkinetic period lasts about 27 minutes in these cells. Under abnormal circumstances cells may become 'blocked' in some stage of mitosis and either fail altogether to go on to the next stage or only do so after a long interval of time. In the flagellate *Holomastigotoides* the intermitotic period is spent in a late prophase condition. Generally, prophase is the longest stage of mitosis; metaphase and anaphase are brief, while telophase is a long stage but not as long as prophase.

1. Prophase

At the beginning of the prophase stage the chromosomes become 'fixable' by ordinary fixing agents – that is to say their appearance in stained microscopical preparations approximates closely to that seen *in vivo* by the most reliable methods of observation. 'Fixability' involves the condensation of the previously diffuse, invisible or dispersed threads into visible chromosomes which are optically double from the very beginning of prophase, i.e. composed of two closely parallel *chromatids*. The fundamental biochemical basis of cellular reproduction – the formation of two genetically equivalent chromatids from a single one – has already taken place before the first microscopically visible indications of mitosis. Thus the stages of cell-division which we can observe and study are not concerned with chromosomal reproduction as such, but only with the distribution of the products of chromosomal reproduction to the two daughter cells. It was at one time believed that the chromosomes were joined together end-to-end to form a continuous loop or 'spireme' at the be-

ginning of prophase, but this is now known to be untrue.

In a diploid nucleus it should theoretically be possible to pick out the homologous chromosomes from the very beginning of prophase, i.e. one ought to be able to arrange the chromosomes in pairs, the members of each pair being the same length. Whether it is in practice possible to do this will depend on whether the several members of the set differ sufficiently in length and on whether they are too long and tangled for analysis.

Usually there is no particular tendency for the homologous chromosomes to be especially close together or far apart in somatic prophase nuclei. But in the Dipterous flies, including *Drosophila*, the homologues usually lie closely parallel throughout prophase and this arrangement ('somatic pairing') is preserved until metaphase, although the homologues are not so closely approximated in the later stages.

In some prophase nuclei the chromosomes describe wide open helices ('spirals') in the nuclear cavity. Such spirals are referred to as *relic spirals*, for a reason that will be apparent later. The direction of coiling of such helices is not constant, i.e. a particular chromosome such as the X-chromosome of the katydid *Tettigonia viridissima* is just as often a right-handed helix as a left-handed one in spermatogonial prophases. In the fern *Osmunda* it has even been shown that the two chromatids of a prophase chromosome may be spiralized in opposite ways.

In prophases fixed and stained with any ordinary chromosomal stain it can as a rule be seen that each chromosome has at least one non-staining gap, which may appear as a constriction or narrow region. There can be little doubt that these are segments of the chromosome which consist mainly or entirely of protein, with little or no DNA or RNA. Where there is

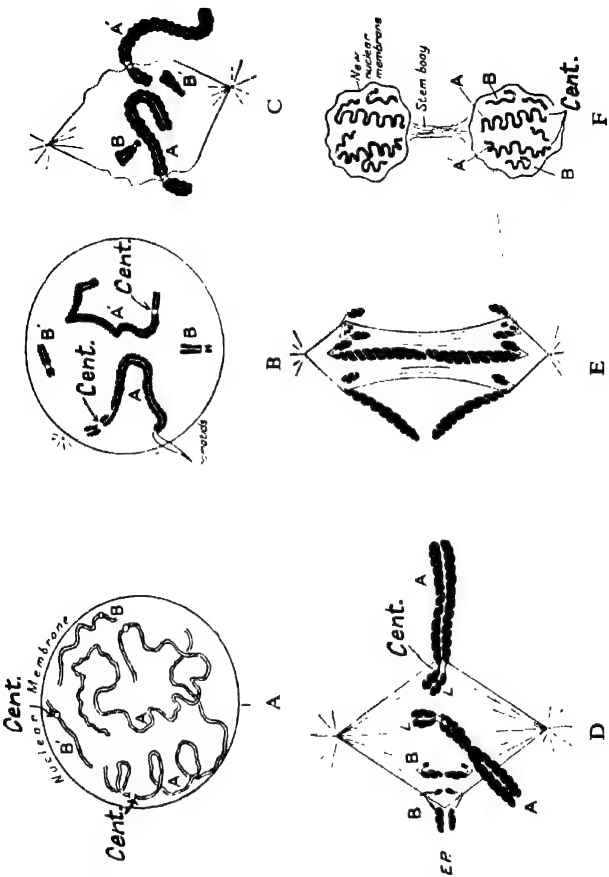


Fig. 2. Diagram of the main stages of mitosis

Only two pairs of chromosomes, A and A′, B and B′, are shown. Both of these have sub-terminal centromeres, those of the B pair being nearer the end. At prophase the relic spirals are clearly seen. *Cent.*, centromeres; *E.P.*, the equatorial plane of the spindle.

only one of these 'constrictions' in a chromosome it marks the position of the *centromere* (or *kinetochore*, as it is called by some cytologists), a special region of the chromosome which plays a vitally important role later on in attaching the chromosome to the spindle. In some chromosomes a small dark staining body or *chromomere* is present in the middle of the centromere constriction, and it has been claimed by some that this is the actual centromere; but since it is not present in all chromosomes this is unlikely.

The centromere may be situated anywhere along the length of the chromosome except that, apparently, it is never quite terminal. But for any particular chromosome (e.g. the human X or the second chromosome of *Drosophila melanogaster*) the position of the centromere is naturally constant, except in so far as it may have been changed by structural changes such as inversions of chromosome segments (see p. 90). It is convenient to distinguish two broad categories of chromosomes, *metacentric* elements in which the centromere divides the chromosome into two approximately equal arms and *acrocentric* ones where the centromere is very close to one end so that one chromosome arm is minute and the other very much longer. Intermediate types exist, however, so that we may describe particular chromosomes as 'subacrocentric' or 'metacentric with one arm twice the length of the other', etc. Some species have all their chromosomes of one type, metacentric or acrocentric, but in many animal species the chromosome set includes both types. Acrocentric chromosomes are generally uncommon in plants, but do exist in a few genera.

Constrictions other than the centromere are sometimes called 'secondary constrictions'. They may be situated anywhere along the length of the chromosome but their positions are ordinarily constant for each

chromosome. They seem to play no special part in relation to the spindle. At prophase it may be impossible to distinguish centromeres from secondary constrictions, since they look alike, but by metaphase the distinction is clear because they have an entirely different relation to the spindle.

In many species of animals and plants one or more *nucleoli* are very obvious during mitotic prophases as bladder-like bodies attached to particular chromosomes (e.g. the X and Y in *Drosophila melanogaster*, the sixth pair in maize, etc.). In the human species there are apparently two nucleoli, one associated with the X-chromosome, the other with an autosome. In all cases nucleoli seem to arise from secondary constrictions; but not all secondary constrictions bear nucleoli. Some species of animals and plants do not show any large nucleoli but may have several or many small structures distributed over the chromosome complement which are probably of the same general nature as nucleoli.

Chemically, nucleoli contain considerable quantities of histone and RNA. There is frequently a tendency for the nucleoli of several chromosomes to fuse together so that one observes a single large spherical nucleolus to which one or more pairs of chromosomes are attached. Nucleoli seem to be formed by specific nucleolar organizers in the chromosomes. When nucleolar organizers are broken into two fragments each piece continues to form a nucleolus. Under various conditions one finds that there is competition between nucleolar organizers for materials used in nucleolus formation, and that some organizers are more potent than others.

Throughout the prophase of mitosis the outlines of the chromatids present a slightly irregular woolly or hairy appearance. They do not in general show a series

of granules (*chromomeres*) such as are seen at the meiotic prophase; this is probably a real difference, and not due to differences in fixability. By the end of prophase the woolly appearance has almost disappeared and a smooth outline has taken its place.

The nuclei of many animals and some algae and fungi have a specialized organelle, the *centrosome* lying just outside it. In the middle of the centrosome there is frequently a single or double dark-staining granule, the *centriole*. The nuclei of higher plants lack centrosomes. In animal cells the centrosome usually divides at the beginning of prophase, its two halves travelling around the nucleus in opposite directions until they are 180° apart. At the same time a system of rays, the *aster*, forms around each of the daughter centrosomes. These may form a conspicuous star-shaped structure on either side of the nucleus and, eventually, at the two poles of the spindle.

2. Premetaphase

At the end of prophase the nuclear membrane usually disappears. In certain Protozoa, however, the whole process of mitosis is intranuclear, the membrane persisting through metaphase. Even in higher organisms it is by no means certain that the nuclear membrane is completely broken down - it may become part of the developing spindle, and later return to its former condition and position at telophase.

The premetaphase stage may be defined as the period during which the spindle is being formed, and during which the chromosomes give the impression of struggling and jostling one another in attempts to reach the equator of the developing spindle.

The spindle is a relatively solid gelatinous body which is apparently composed almost entirely of protein, with a very small amount of RNA.

By the use of rather gentle detergents such as digitonin it has been possible to 'dissolve' away the remainder of the cell and obtain isolated spindles with their attached chromosomes. By repeated washing and centrifugation a sediment of spindles can be obtained. When one uses cells with centrosomes and asters these remain attached to the spindles isolated by this method.

Various drugs such as colchicine can be used to interfere with spindle formation and may be said to act as spindle poisons. Usually they also cause over-condensation of the chromosomes. An abnormal 'C-mitosis' occurs in which anaphase is entirely omitted, so that both groups of daughter chromosomes are included in the same nucleus. Such drugs are used in plant breeding in order to produce polyploid individuals.

There is a bewildering variety of spindle types, which have never been adequately classified. Some spindles are elongated and pointed at the poles, others are more barrel-shaped and may be truncated at the ends. In most, but by no means all Metazoa, a centrosome is present at each pole, sometimes with astral rays radiating out from it into the cytoplasm. Some animals have spindles that flare out at the end rather than coming together in a pointed pole; such spindles are characteristic of the first meiotic division in some insects. In some instances one can hardly speak of a single spindle at all, each chromosome or chromosome pair (at meiosis) having an independent spindle element or component. Under abnormal circumstances, for example if more than the usual number of chromosomes are present, we may get multipolar spindles formed, with three, four or more 'poles'. In certain insects so-called unipolar spindles are formed at meiosis. It is not clear whether these cone-shaped bodies should be regarded as having one pole only or as having two poles, one pointed, the other diffuse.

Where centrosomes are present they seem to act as organizers of the spindle which forms between them. The birefringence of the spindle indicates that its molecules are orientated in the direction of the long axis. In some instances spindle fibres can be seen – not only in fixed preparations but even in the living cell. These are probably bundles of parallel protein molecules. A distinction may be made between continuous fibres which run from pole to pole and chromosomal fibres which connect the centromeres to the poles.

In the case of bipolar spindles one can recognize an equatorial plane perpendicular to the long axis and midway between the poles. In the case of multipolar spindles we have several equatorial planes which intersect one another. As premetaphase proceeds the chromosomes arrange themselves with their centromeres on the equatorial plane; when they have done so we may consider that the dynamic stage of premetaphase is at an end and that the relatively static metaphase stage has begun.

3. Metaphase

At the end of premetaphase the chromosomes have reached their maximum degree of condensation and have acquired smooth outlines. Each element consists of two chromatids lying parallel or loosely wound around one another and physically united only at the centromere, which behaves as if it were undivided (more probably it has undergone replication in a chemical sense at the same time as the rest of the chromosome but without separation of the 'daughter centromeres').

Although the fact is not as a rule visually apparent, evidence from many sources indicates that each chromatid is a closely coiled helix. Special techniques may be employed for demonstrating the spiral structure of chromosomes at metaphase, particularly in the case of

some plant species with very large chromosomes. In some organisms the chromatids are uniform in diameter throughout, i.e. cylindrical; while in others the chromosomes at metaphase are slightly club-shaped, their diameter being least in the region of the centromere. Nucleoli have usually disappeared by this stage, their

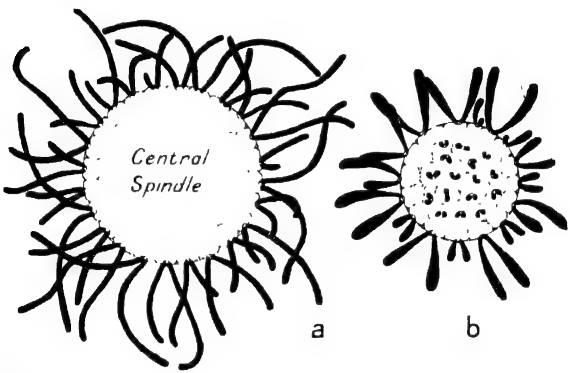


Fig. 3. Semidiagrammatic polar views of chromosomes and spindles at mitotic metaphase

a, in an organism such as a Urodele in which all the chromosomes attach themselves to the periphery, leaving a large 'central spindle' free of chromosomes. *b*, in a reptile with 16 macrochromosomes which arrange themselves around the periphery of the spindle and 16 microchromosomes which occupy the central region.

substance having apparently contributed to the volume of the condensing chromosomes.

Where there is a considerable range in the size of the chromosomes the larger elements almost always occupy the periphery of the equatorial region of the spindle, their long arms projecting out of the spindle into the cytoplasm; while in others the distribution of the

chromosomes at metaphase is more nearly random. In some species with all their chromosomes relatively long, one finds metaphases in which the chromosomes lie in a ring, the central region of the spindle having no chromosomes ('hollow spindle').

Metacentric chromosomes normally appear V-shaped at metaphase, while acrocentrics appear as straight or slightly curved rods. The centromeres appear to be regions where the chromosome is more flexible than elsewhere.

4. Anaphase

Metaphase is a stage during which little or no visible change takes place in the cell: it is a static phase and usually a short one. The beginning of anaphase is marked by a separation of the daughter centromeres from one another. One gets the impression that the centromeres suddenly split and that their halves actively repel one another (whether this is a correct interpretation of what is happening is another matter). As the daughter centromeres move up the spindle towards the poles they drag the chromatids which are attached to them apart from one another. Mitotic separation of chromatids thus always starts at the centromere and proceeds along the chromosome in both directions. As soon as the chromatids have entirely separated from one another they are more appropriately called *daughter chromosomes*.

At the same time as the daughter centromeres are separating from one another, the equatorial region of the spindle between them is elongating. The poles of the late anaphase spindle are hence considerably further apart than those of the metaphase spindle. The relative importance of the two processes – autonomous movement of the centromeres and spindle elongation – varies from one organism to another.

Where the equatorial part of the spindle elongates very considerably it becomes correspondingly narrowed and eventually appears as a thin bundle of thread-like structures between the two separating groups of daughter chromosomes – the so-called *stem body.*

In a few cases – the meiotic divisions of moth eggs and the cleavage divisions in the embryos of certain mites are the classic examples – masses of 'chromatin' material are left behind on the equator of the spindle as the daughter chromosomes pass to the poles. These masses correspond in number and general dimensions to the individual chromosomes, so that each one is apparently sloughed off in the course of division. These masses are Feulgen-negative and hence probably consist of ribose nucleoprotein. They may correspond to the chromosomal 'matrix' recognized by many of the earlier cytologists – a concept which should probably not be discarded entirely, although some of the properties formerly attributed to the 'matrix' clearly went considerably beyond the evidence.

5. Telophase

As a rule the two groups of daughter chromosomes do not actually reach the poles of the spindle, although as a result of the elongation of the stem-body they may travel further apart than the original distance between the poles at metaphase. The polar caps of the spindle seem to be destroyed at the beginning of telophase, but the stem-body may persist for a long time, even after cell-division has been completed. The two groups of daughter chromosomes rapidly lose their smooth outlines and begin to undergo de-condensation. They are usually in a tangled mass at this stage and an apparently new nuclear membrane forms around them. Broadly speaking we may describe the changes that occur in the chromosomes at telophase as the reverse of those that

have taken place at prophase; for example nucleoli reappear at the same sites where they disappeared at the end of prophase. But each telophase chromosome consists of only one chromatid instead of two, and there is only half the amount of DNA, so that the appearance of prophase and telophase nuclei in the same tissue is almost always characteristically different. The de-condensation of the chromosomes during telophase seems to involve a relaxation of their spiral structure: but the coils are not lost entirely and may persist through the interphase to reappear as the relic coils at the prophase of the next division. The details of cytokinesis, the process whereby the cytoplasm becomes divided into two daughter cells, lie outside the scope of this book. Usually the two cells produced by mitosis are equivalent, but in some instances they differ more or less profoundly. Mitoses which lead to the formation of two visibly different daughter cells may be referred to as *asymmetrical divisions*. Usually, in such cases the main difference is in the amount of cytoplasm allocated to the two cells at the end of the division, one daughter cell being much larger than the other. The two nuclei may look alike at telophase but become visibly different in appearance as they return to the resting condition, although they contain genetically equivalent chromosome sets.

The dynamics of the mitotic process

No entirely satisfactory dynamic interpretation of the entire spindle mechanism has been put forward. The formation of the spindle at the end of prophase would seem to involve two processes: (1) the aggregation of similar molecules, with exclusion of cytoplasmic granules and other 'foreign bodies' (including even pieces of chromosome lacking centromeres, if these are present), and (2) the orientation of these molecules into

a 'biological crystal'. These processes may both be expressions of a single influence emanating from the centromeres and (where these are present) the centrioles. Various kinds of 'fibres' have been recognized in spindles. SCHRADER distinguishes between *continuous fibres* which connect the two poles directly, *chromosomal fibres* which connect the centromeres to the poles, and *interzonal connexions* which run between the tips of the separating daughter chromosomes at anaphase.

There has been considerable argument as to the 'reality' of these various kinds of fibres. At least in one type of cell, the spermatocytes of the mite *Pediculopsis*, the continuous fibres have been seen *in vivo* by ordinary light. Some other living spindles appear to be structureless and only become visibly fibrous on fixation. No doubt exists that the spindle consists largely of elongated molecules lying parallel to its long axis. This interpretation is firmly supported by observations of its birefringence and studies of the effects of dehydration, which shrinks the spindle in width rather than in length. The argument as to just how 'real' the spindle fibres are is hence concerned with the rather narrow issue as to how far the fibrous structure of the fixed spindle is prefigured in life by special tracts of orientated molecules.

The earliest interpretation of the anaphase movement was that it was caused by an actual contraction of the chromosomal fibres, which pulled the daughter chromosomes apart. It is known that a decrease in birefringence occurs during anaphase, and that it spreads from the equator towards the poles. Some observations suggest that an active substance (possibly a proteolytic enzyme?) is liberated by the centromeres at this stage. As the equatorial region of the spindle elongates to form the stem body at late anaphase it is almost impossible not to draw the conclusion that the daughter chromosomes

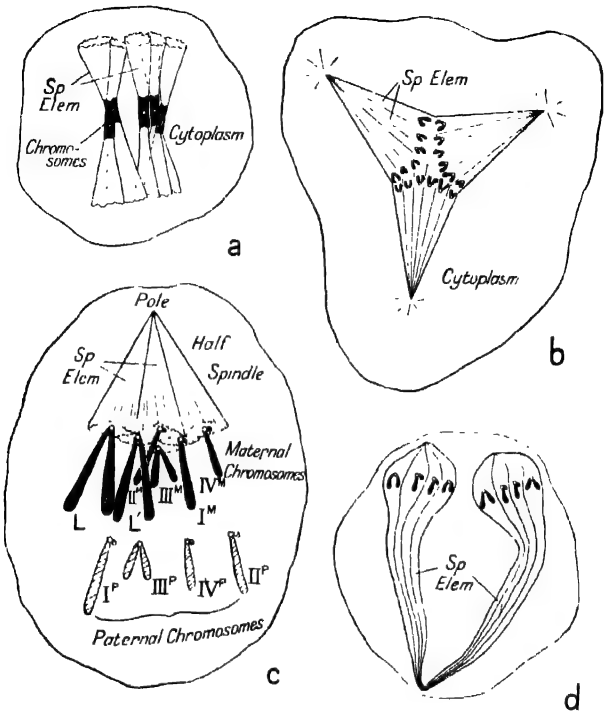


Fig. 4. Various unusual types of spindles

a, the spindle of the first meiotic division in the scale insect *Llaveia bouvari*, where the spindle elements are separate and flare out towards the 'poles' (after Hughes Schrader). *b*, the tripolar spindle at early anaphase. *c*, the half-spindle formed at the first meiotic division in the spermatogenesis of the fly *Sciara coprophila* (after Metz); all the maternal chromosomes, including the two 'limited' ones L and L′, go to the pole, the paternal ones moving in the opposite direction. *d*, a late anaphase spindle of the first meiotic division in the abnormal spermatogenesis of an F_1 hybrid between *Drosophila pseudoobscura* and *D. persimilis*.

are being actively pushed apart. But the precise nature of the forces involved in the earlier phase of anaphase separation is much more uncertain.

It was formerly believed that many types of cells divided by a process known as *amitosis*, involving elongation of the nucleus into a dumbbell-shape and eventual formation of two nuclei, without any formation of a spindle and indeed without any precise separation of equivalent daughter chromosomes. Most of the earlier reports of amitotic divisions were probably based either on pathological mitoses or on normal mitoses in which severe clumping of the chromosomes had occurred as a post-mortem artifact. The division of the ciliate macronucleus is certainly amitotic in a sense, but these are highly polyploid nuclei and it is by no means certain that they lack an intranuclear mechanism which ensures that daughter nuclei receive equivalent sets of chromosomes.

In recent years we have learned a great deal about some of the chemical processes involved in mitosis. But, as MAZIA has put it: 'Mitosis cannot be understood merely as a series of chemical transformations. It is a dynamic mechanical process, a matter of push and pull, stress and strain.'

CHAPTER III

Number, Form and Size of Chromosomes

In general, the chromosome set or *karyotype* is constant for the somatic cells of the individual, and for all individuals of the species. Numerous exceptions to both of these statements exist, however (i.e. on the one hand, individuals with different chromosome numbers in the various cells of the body, and on the other, species which include individuals with different chromosome numbers). These special cases will be considered in detail later.

The basic number of chromosomes in the somatic cells of an individual is referred to as the *somatic number*. Certain cells and tissues may contain multiples of this number and are referred to as *polyploid*. Thus if the basic number is x, some somatic cells may contain $2x$, $4x$, $8x$, $16x$ . . . , as a result of repeated doubling of the basic number.

Where there are size differences between the chromosomes of a somatic set, one can often see that they consist of a number of pairs, the members of each pair being alike in size, centromere position and other special features such as heterochromatic regions, nucleolar and secondary constrictions, etc. Individuals and species in which the chromosomes can be 'paired up' in this way are referred to as *diploid*, and the somatic set is then a *diploid set*, which may be regarded as

made up of two *haploid sets*. The vast majority of animal species, and about half the species of higher plants, are diploid. In a few animal species, however, and in a great many higher plants, the chromosomes can be grouped, not into pairs, but into threes, fours or groupings of higher numbers. Such organisms (in which the somatic number is not diploid) are called *polyploids*, those with 3 of each kind being *triploids*, those with 4 of each kind *tetraploids* and so on (*pentaploids*, *hexaploids*, *heptaploids*, *octaploids*, etc.). Occasionally we meet with individuals which have only one of each kind of chromosome and are consequently *haploids*. Polyploids in which all the chromosome sets have been derived from a single species are called *autopolyploids*, while those which have been derived from interspecific hybrids, and hence contain chromosome sets derived from two or more species, are called *allopolyploids*.

Even in diploid organisms one pair of chromosomes may be unequal in size or shape, in one sex but not in the other. In such cases we have to do with *sex chromosomes*, which play a special role in the determination of sex. In extreme cases the inequality between the two kinds of sex chromosomes may be carried to the point where one of them is actually non-existent. In such species the diploid or somatic number in one sex is an odd one, and the two haploid sets differ in respect of the presence or absence of one chromosomal element.

In addition to differences between the two haploid sets which are due to sex chromosomes, there are some diploid individuals in which other chromosomal differences, not associated with sex, exist between the two haploid sets. Thus the concept of diploidy does not require that the two haploid sets should be identical, either in a cytological sense or in genetic content, but merely that they should be similar. In sexually reproducing diploids one haploid set will have been derived

from one parent and one from the other, but parthenogenetic diploid species exist in which both haploid sets will have been inherited from the mother, there being no male parent in such species.

The two chromosomes of a pair are said to be homologous, since genetic evidence indicates that, in general, they contain the same series of genetic loci arranged in the same order. In triploids there will be 3 homologues of each kind, in tetraploids 4 homologues, and so on. The concept of genetic homology may be applied to parts of chromosomes as well as to whole ones, since one sometimes finds a pair of chromosomes which are homologous in some regions but not in others (see below). Partial homology is especially characteristic of individuals which are hybrids between two different species, since the haploid sets derived from the two parent species will be dissimilar in various ways.

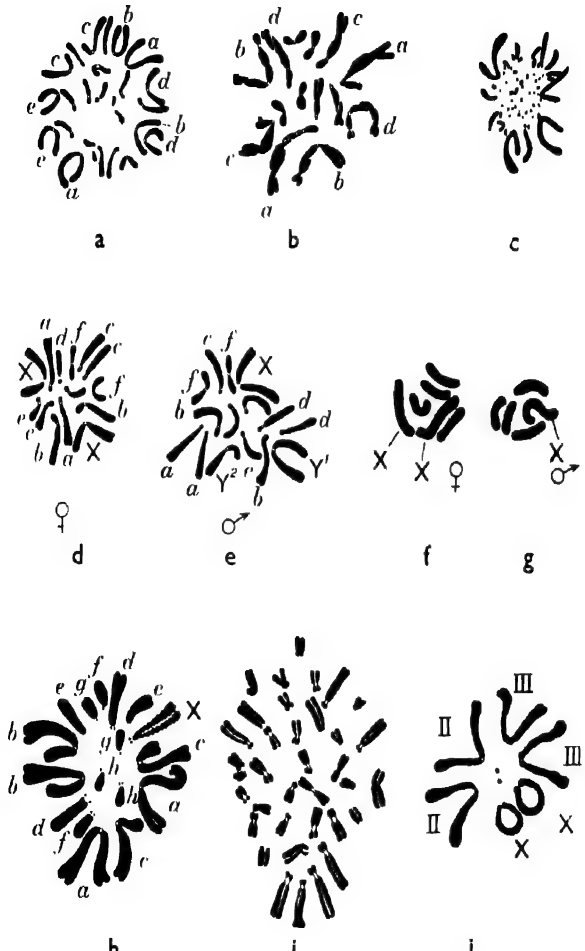
The lowest conceivable haploid number is, of course, 1, which occurs in the horse nematode *Parascaris equorum* (= *Ascaris megalocephala*), or at any rate in certain strains of this species, since other strains (referred to as variety *bivalens*) have a haploid number of 2 (diploid number = 4). Actually, these numbers refer only to the cells of the germ-line (fertilized egg, primordial germ cells, spermatogonia and oogonia). In the somatic cells of *Parascaris* a much larger number of chromosomes are found, as a result of fragmentation of the two or four large chromosomes originally present in the fertilized egg (see later). Very few animal species (some mites, a few midges, certain scale insects) have a haploid number of 2 and recently a higher plant (*Haplopappus gracilis*) has been found with the same number. Species with a haploid number of 3 are a little more common and occur in many groups. The haploid numbers of most diploid species of animals and plants lie between about 6 and 25, higher or lower numbers

being uncommon except in a few taxonomic groups. The highest haploid number hitherto recorded is n = ca. 510 in the fern *Ophioglossum petiolatum*, but this species is certainly a very high polyploid (possibly about 32-ploid), so that its 'haploid' number is a derived one, and is not the 'basic' haploid number. As far as animals are concerned the highest chromosome number known is in a little butterfly from Spain, *Lysandra nivescens*, with 190 or 191 chromosomes in the haploid set; this species is almost certainly *not* a polyploid. Highly polyploid cells in individuals with quite moderate chromosome numbers may contain many thousands of chromosomes, possibly hundreds of thousands in some instances.

Among animals the Diptera are probably the outstanding example of a group with low chromosome

Fig. 5. Chromosome sets of various organisms (diploid, except for i, *which is the haploid complement)*

a, a frog, *Rana temporaria*, with $2n$ = 26 (after Makino, 1932, *Proc. Imp. Acad.* **8**: 23). *b*, the plant *Puschkinia libanotica*, showing chromatids spirally wound round one another ($2n$ = 10 plus 4 supernumerary chromosomes) (after Darlington, 1937, fig. 5). *c*, chicken, *Gallus domesticus*, showing extreme size range (original). *d* and *e*, the two sexes of the Dock, *Rumex acetosa*, showing the sex chromosomes and the subacrocentric autosomes (after Kihara and Yamamoto, 1932, *Cytologia* **3**: 84). *f* and *g*, the scale insect *Llaveia bouvari*, a species with XO males and no discernible centromeres (probably polycentric condition) (after Hughes Schrader, 1931, *Z. Zellforsch.* **13**: 742). *h*, spermatogonial division in the grasshopper *Chorthippus parallelus* ($2n$ ♂ = 17), showing the negative heteropycnosis of the X-chromosome and the extremely small size of the short arms of the acrocentric elements (original). *i*, pollen grain mitosis in the plant *Gagea lutea*, with n = 36 (after Matsuura and Suto, 1935, *J. Fac. Sci. Hokkaido Univ.* V, **5**, fig. 167). *j*, female *Drosophila melanogaster*, with two ring-X's (after Morgan, 1933, *Genetics* **18**: 250). Figures of other authors redrawn.


a
b
c
d
e
f
g
h
i
j
X
Y
Y'
Y²
♀
♂
II
III

numbers ($n = 2$ to 9, modal numbers 4 and 6). Another group of the same type is the Rhabdocoel flatworms ($n = 2$ to 10, modal number 2). At the other end of the scale we have groups like the Decapod Crustacea and the birds, where all the species that have been studied have high numbers ($n > 25$). The Lepidoptera show a great range of chromosome number (from $n = 8$ to $n = 190$ or 191) but the modal number is 31, so that this is a group with predominantly high numbers. In the

TABLE II

Diploid numbers of some organisms frequently used in biological, agricultural and medical work

THALLOPHYTA

Aspergillus nidulans 8?
Neurospora crassa 14
Saccharomyces cerevisiae 10 or 12

HIGHER PLANTS

Pinus spp., *Larix* spp., *Abies* spp. 24
Juniperus spp. 22
Arabidopsis thaliana 10
Brassica oleracea (Cabbage and cult. vars.) 18
Raphanus sativus (Radish) 18
Linum usitatissimum (Flax) 30 32
Lythrum salicaria 30, 60 (diploid and tetraploid)
Oenothera lamarckiana (Evening Primrose) 14
Epilobium spp. 36
Cucumis sativus (Cucumber) 14
Citrullus vulgaris (Water Melon) 22
Carica papaya (Papaya) 18
Eucalyptus spp. 22
Gossypium hirsutum and *G. barbadense* (Upland and Sea Island Cotton) 52 (tetraploids)
Prunus domestica (Plum) 48 (hexaploid)
Acacia spp. 26 and 52 (diploid and tetraploid)
Arachis hypogaea (Peanut) 40 (tetraploid)
Phaseolus vulgaris (Bean) 22
Pisum sativum (Garden Pea) 14
Vicia faba (Field Bean) 12

TABLE II (*contd.*)

Trifolium repens (White Clover) 32 (tetraploid)
T. subterraneum 16
Nicotiana tabacum (Tobacco) 48 (tetraploid)
Lycopersicum esculentum (Tomato) 24
Solanum tuberosum (Potato) 48 (tetraploid)
Camellia sinensis (Tea) 30
Coffea arabica (Coffee) 44 (tetraploid)
Quercus spp. (Oaks) 24
Musa paradisiaca (Banana) 22, 44, 55, 77, 88
Allium cepa (Onion) 16
Zea mays (Corn) 20
Hordeum vulgare (Barley) 14
Triticum aestivum (Bread Wheats) 42 (hexaploid)
Secale cereale (Rye) 14
Oryza sativa (Rice) 24 (tetraploid)
Saccharum officinarum (Sugar Cane) 80 (octaploid)
Avena sativa (Oats) 42 (hexaploid)
Tradescantia virginiana 24 (tetraploid)

ANIMALS

Drosophila melanogaster 8
Musca domestica (Housefly) 12
Mosquitoes (many species) 6
Apis mellifica (Honey Bee) 32
Bombyx mori (Silkworm) 56
Grasshoppers (most species of Acrididae) 23 (♂), 24 (♀)
Grouse Locusts (Tetrigidae) 13 (♂), 14 (♀)
Helix pomatia (Roman Snail) 54
Cepaea nemoralis 44
Ambystoma mexicanum (Axolotl) 28
Triturus (European spp.) 24
T. viridescens (U.S.) 22
Rana spp. (Frogs) 26
Hyla spp. (Tree Frogs) 24
Bufo spp. (Toads) 22
Gallus domesticus (Chicken) ca. 78
Meleagris gallopavo (Turkey) 82
Columbia livia (Pigeon) 80
Anas platyrhyncha (Duck) 80
Mus musculus (Mouse) 40
Rattus norvegicus and *R. rattus* 42
Mesocricetus auratus (Golden Hamster) 44
Cricetulus griseus (Chinese Hamster) 22

TABLE II (*contd.*)

Cavia cobaya (Guinea Pig) 64
Oryctolagus cuniculus (Domestic Rabbit) 44
Macaca mulatta (Rhesus Monkey) 42
Homo sapiens 46
Canis familiaris (Dog) 78
Felis domestica (Cat) 38
Equus caballus (Horse) 66
E. asinus (Donkey) 66
Sus scrofa (Hog) 40
Ovis aries (Sheep) 54
Capra hircus (Goat) 60
Bos taurus (Cattle) 60

mammals there is a rather clear distinction between the Marsupials, with low chromosome numbers ($n = 6$ to 14), and the Eutheria, with much higher numbers ($n = 9$ to 39, but over 20 in the great majority of species). In the plant kingdom groups such as the ferns and horsetails, with many polyploid species, naturally show high chromosome numbers, while groups like the Fungi and Gymnosperms, where polyploidy is very rare, show much lower numbers.

STEBBINS refers to karyotypes in which all the chromosomes are approximately the same size and are all equal armed metacentrics as *symmetrical*; *asymmetrical* karyotypes being ones which include elements of different sizes or in which some of the chromosomes are J-shaped or acrocentric. As far as the higher plants are concerned he regards symmetrical complements as primitive, asymmetrical ones as derivative.

It is rather difficult to apply these concepts in the animal kingdom, where extremely asymmetrical karyotypes are almost universal in many groups such as grasshoppers, the genus *Drosophila*, reptiles and birds. In some ways it seems better to consider range of chromosome size and range of arm ratios as quite separate variables.

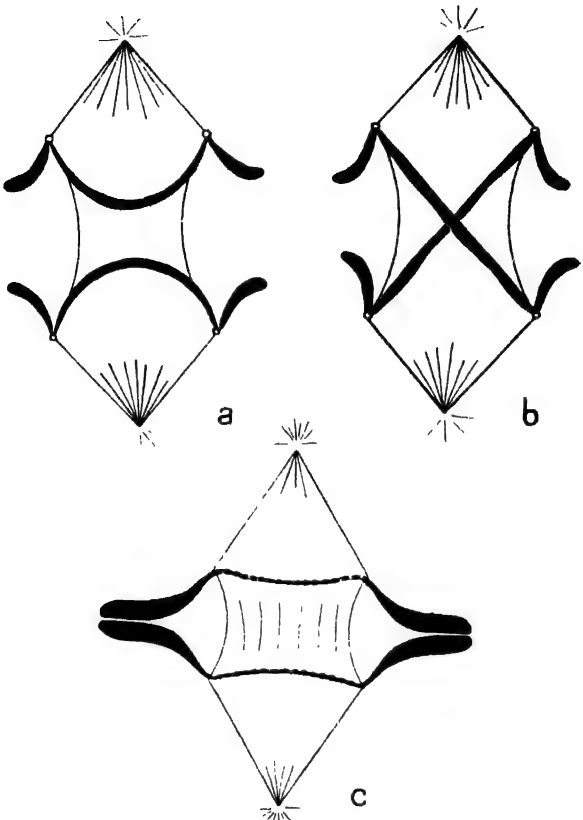


Fig. 6. a *and* b, *diagrams to show the alternative ways in which a dicentric chromosome may behave at anaphase.* c, *diagram of the method of anaphase separation in one of the germ-line chromosomes of the nematode* Parascaris equorum*; the numerous centromeres are situated so close together that those in one chromatid always go to the same pole*

In most taxonomic groups of animals and plants each chromosome possesses only a single centromere. Chromosomal rearrangements, either spontaneous or radiation-induced, may create new types of chromosomes which either lack a centromere or possess two of them. The former (*acentric* chromosomes) do not become attached to the spindle at mitosis and tend to get left out in the cytoplasm at telophase, where they eventually degenerate as a rule. The latter (*dicentric* chromosomes) may pass through a series of cell-divisions without mishap; eventually, however, it may happen that the two centromeres of each daughter chromosome pass to opposite poles at a particular anaphase (rather than to the same pole). If this occurs, a double chromosomal 'bridge' will be formed between the two separating daughter nuclei. It may prevent their separation (in which case a binucleate cell or a nucleus with twice the original chromosome number will be formed). Alternatively, the 'bridges' may break under the strain, in which case each of the daughter nuclei will contain two freshly broken ends. If these fuse again (as they will in most cases) a new dicentric chromosome will be created, and the series of accidents will be repeated. This kind of behaviour has been called the 'chromosome type of breakage-fusion-bridge cycle'. It is undoubtedly the reason why dicentric chromosomes do not normally occur in nature, and why they cannot be maintained in laboratory stocks of most organisms.

The above interpretation, although generally valid, does not apply in certain special cases. One is the horse nematode *Parascaris equorum*, already referred to, in which the central regions of the long germ-line chromosomes undergo fragmentation in certain of the embryonic divisions which are destined to give rise to somatic cells. In this instance it is fairly clear that the germ-line chromosomes possess rather numerous centromeres in

their middle regions, so that when fragmentation occurs each piece receives at least one centromere. The end portions of the germ-line chromosomes do not seem to contain any centromeres and are cast off during the fragmentation-mitoses, behaving as acentric elements. In spite of the fact that the germ-line chromosomes are *polycentric* all the centromeres of each daughter chromosome seem to regularly pass to the same pole at anaphase, so that the chromosomes do not form bridges at anaphase and are not liable to be disrupted in the germ-line mitoses. In some other species of Ascarid worms the ends of the chromosomes are cast off in the somatic cells in the same manner as in *P. equorum*, but there is no fragmentation of the middle regions of the chromosomes.

A second type of instance where it cannot be said that each chromosome has a single centromere occurs in certain Homopterous insects (particularly scale insects and aphids), probably also in the scorpions *Tityus* and *Isometrus* (but not in all scorpions), certainly in rushes of the genus *Luzula*, possibly also in the alga *Spirogyra* and very likely in a number of other taxonomic groups. In none of the above-cited organisms can centromeres be detected as individualized, microscopically visible entities. Nor are the chromosomes bent at metaphase or anaphase in a manner which would indicate a localized centromere. Characteristically, the two daughter chromosomes remain parallel, like a pair of stiff rods, as they separate at anaphase. In some instances breakage of these chromosomes by radiation has been shown to give rise to several fragments, all of which become attached to the spindle and divide normally in subsequent mitoses. It has been suggested that in these various organisms the chromosomes possess a 'diffuse centromere activity', which extends along their entire length. Alternatively they may possess numerous centromeres,

perhaps each of very small size, distributed along their length. Chromosomes of this type may perhaps be more widely distributed in the animal and plant kingdoms than has generally been recognized in the past.

We have already referred to the tendency of freshly broken chromosome ends to fuse together. It does not seem to matter whether the breakage is spontaneous, due to irradiation or to mechanical tension on the spindle – in all cases it gives rise to two freshly broken ends or surfaces which behave as 'sticky'. Natural chromosome ends do not show this tendency to fuse with other ends and are hence non-sticky. The term *telomere* has been used to designate the natural chromosome ends. Some doubt still exists as to whether telomeres are actually visible in some instances as discrete bodies at the ends of the chromosomes, but the term is justified by the special behaviour of the chromosome ends, even if no individualized structure can be seen. It has recently been suggested that telomeres may consist essentially of a special kind of temporary fusion between the ends of sister strands (chromatids or subchromatids).

In the Ascarid worms the status of telomeres is rather uncertain, since interstitial portions of the germ-line chromosomes become terminal, after fragmentation in the somatic nuclei.

In certain laboratory stocks of *Drosophila*, Maize and other organisms it has been possible to obtain ring chromosomes, i.e. chromosomes with no ends, which have a centromere but no telomeres. Some of these are fairly stable, and can pass through a series of mitoses without accident, giving rise to two daughter rings at each division. Others are unstable because they give rise to double-sized dicentric rings which undergo a special kind of breakage-fusion-bridge cycle. Ring chromosomes do not seem to have established themselves in

the normal karyotype of any species of plant or animal.

The smallest chromosomes are about 0·25 of a micron long at metaphase and about the same breadth; this is close to the limit of resolution of the light microscope. It seems unlikely, but perhaps not impossible that some organisms may possess chromosomes that are actually too small to be seen even with the best light microscopes. In most fungi all the chromosomes are extremely minute, the whole nucleus being barely visible. Other examples of very small chromosomes occur in the birds (where there are also some fairly large elements in the karyotype).

The longest mitotic chromosomes known (apart from the salivary gland chromosomes of Diptera, which are not really 'mitotic') are probably those of the plant *Trillium* which may reach 30 micra in length. Other plants with conspicuously large chromosomes include many Liliaceae and some but not all Commelinaceae (*Tradescantia* and related genera). Among the animals the orthopteroid groups of insects (grasshoppers, crickets, praying mantids, etc.) and the Urodeles (newts and salamanders) have particularly large chromosomes, although not so large as those of *Trillium*. The mitotic chromosomes of *Drosophila* are only about 3·5 micra in length, and those of man average about 5 micra long.

In general, all individuals of the same species will exhibit the same chromosome number, except where the number of sex chromosomes is different in the two sexes. Rather numerous examples are known, however, in which the chromosome number of a species is variable. Some of these variations may be due to supernumerary chromosomes (see page 147) which are not essential for life and may exist in different numbers in the individuals of a population. Others are due to various types of chromosomal rearrangements which

have occurred and whose effect is, roughly, that a single chromosome in one individual is the genetic equivalent of two separate elements in another.

A good many of the early determinations of chromosome numbers were inaccurate, due to faulty technique. In certain groups with large and relatively few chromosomes almost any technique, however crude, is adequate for an accurate determination of the number and gross morphology of the chromosome complement. In such groups even the cytological accounts of 50 years ago are essentially accurate (grasshoppers, salamanders and lilies will serve as examples). In other groups such as the birds the chromosome numbers are very high, many of the elements are close to the theoretical limit of resolution of the light microscope and - worst of all – the metaphase chromosomes have a strong tendency to fuse or clump together into aggregates, unless special precautions are taken, and even with the best techniques clumping is never entirely eliminated, so that the true chromosome complement is only revealed in a minority of the metaphases in the preparation. Such technical difficulties account in part for the fact that until very recently it was believed that the diploid number in the human species was 48 when, in fact, it is certainly 46. In the latter case the obvious difficulty of obtaining meiotic material and the fact that minor variations in chromosome number do seem to exist from cell to cell in certain of the somatic tissues also contributed to the confusion.

Even with the most difficult vertebrate materials, however, the standard of modern descriptive chromosome cytology is now very high and few or no errors seem to exist in the recent accounts by experienced workers. Recent claims that the germ-line chromosome number of normal members of the human species is variable and that the smaller elements in the central part

of the spindle in the case of the chicken are not true chromosomes at all cannot, however, be regarded as securely founded. Uncertainties of chromosome number may exist where the number is a very high one indeed, e.g. in the case of some polyploid ferns; or where relatively high numbers of extremely minute chromosomes are present.

Heterochromatin and heteropycnosis

In most species of animals and plants it can be seen at various stages of mitosis and meiosis that certain chromosomes or segments of chromosomes are more (or in some cases less) condensed than the rest of the karyotype. This phenomenon has been called *heteropycnosis* ('different thickening'). We may distinguish between positive heteropycnosis ('over-condensation') and negative heteropycnosis ('under-condensation'). However, the same chromosome or chromosome region may exhibit positive heteropycnosis at one stage of its cycle and no heteropycnosis or even negative heteropycnosis at another stage. Chromosomal material which shows heteropycnosis at some stage is referred to as *heterochromatin*, the 'standard' regions which do not show heteropycnosis being *euchromatin*.

Genetic evidence has shown that heterochromatic chromosomes and regions contain relatively few active genes in relation to their length. Thus the Y-chromosome of *Drosophila melanogaster*, which is quite a long element but entirely heterochromatic, is relatively inert, genetically. Male flies lacking a Y are viable, so that it does not contain any genes essential for life. They are, however, sterile; and it is known that several genetic loci in the Y must be present in order that normal sperms can be formed. Another example of a type of chromosome which is largely composed of heterochromatin and almost completely inert genetically, is provided by the

so-called B-chromosomes of maize. These are supernumerary chromosomal elements which may be present in varying numbers in certain varieties, without visibly affecting the appearance of the plant (if too many of them are present they may produce deleterious effects on viability). Similar supernumerary chromosomes are present in the natural populations of many species of animals and plants; in all cases the individual can exist quite well without them, although they presumably do have some adaptive properties. In certain species of plants the supernumerary chromosomes become lost from the somatic cells, in the course of development, but are retained in the germ-line.

In most species of animals all the chromosomes have heterochromatic regions around the centromeres, and in many cases there are also distal heterochromatic segments at the ends of the chromosomes. It seems quite possible that all chromosomes contain both euchromatic and heterochromatic segments, although those of some plant species have been reported to lack heterochromatin.

Some species of animals show two quite distinct kinds of heterochromatin, a 'compact' and a 'diffuse' type. In such cases the nucleolar organizer always seems to be located in the diffuse heterochromatin. It seems probable that what we ordinarily refer to as heterochromatin and euchromatin are only the end members of a continuous series and that intermediate types of 'chromatin' also exist. Obviously, some general chemical difference between heterochromatin and euchromatin must exist but its nature is not understood. As a tentative hypothesis we might suggest that euchromatic DNA consists of adenine-thymine and cytosine-guanine nucleotide pairs arranged in a great variety of sequences, while heterochromatic DNA consists largely of one kind of nucleotide pair only (either A-T or C-G). If so, 'compact' heterochromatin might consist in the main of A-T

nucleotide pairs and 'loose' heterochromatin of C-G pairs (or vice versa). While purely hypothetical at the present time, such a hypothesis would seem in accord with the rather unspecific and indistinct genetic properties of heterochromatin and with the tendency of all heterochromatic segments to undergo pairing at meiosis or in the salivary gland nuclei of the Diptera (see p. 54).

In short-horned grasshoppers (Acridoidea) and crickets the X-chromosome generally shows a 'reversal of heteropycnosis' in the course of spermatogenesis, being negatively heteropycnotic in the early spermatogonial divisions and positively heteropycnotic during the prophase of the first meiotic division. In many species this reversal is followed by a second one; the X-chromosome becomes negatively heteropycnotic in the first meiotic metaphase and anaphase and then positively heteropycnotic again in the spermatid. The heterochromatic regions in the autosomes of these species do not, as a rule, show this reversal of behaviour and never show negative heteropycnosis. In the long-horned grasshoppers (Tettigonioidea) even the X-chromosome does not seem to exhibit negative heteropycnosis.

In certain species of plants there are special regions of the chromosomes which show up as negatively heteropycnotic or under-condensed segments in tissues that have been kept alive at low temperatures for some time before fixation. This phenomenon has been interpreted as a direct effect of 'nucleic acid starvation' at low temperatures. Some determinations of the total amount of DNA present have, in fact, shown that there is slightly less in these cold-treated nuclei; but the whole matter is rendered rather confused by the fact that heat-treated nuclei (which do not show under-condensed regions) also appear to show a sub-standard quantity of DNA.

Endopolyploidy and endomitosis

In many adult differentiated tissues in which the cells have ceased dividing by mitosis the nuclei no longer contain the original 'somatic' number of chromosomes but 2, 4, 8, 16 . . . times that number. This phenomenon, termed *endopolyploidy*, has been studied especially in the somatic tissues of insects and in some of the higher plants; but it is known to exist in other groups such as the vertebrates and will no doubt eventually be demonstrated in most or all higher organisms (i.e. above the level of fungi and protozoa). Even in the latter group the macronuclei of the Ciliates are certainly endopolyploid.

Endopolyploidy arises through a process (termed *endomitosis*) whereby all the chromosomes in a 'resting' nucleus reproduce simultaneously, the daughter chromosomes separating from one another within the intact nuclear membrane, without the formation of any spindle or mitotic apparatus in the ordinary sense. In certain types of endomitosis there seems to be a cycle of condensation and de-condensation of the nucleoprotein material of the chromosomes and some cytologists have distinguished stages such as 'endoprophase', 'endometaphase' and 'endotelophase'. But such changes in the degree of condensation of the chromosomes do not seem to be essential to the mechanism of endomitosis, since they do not always occur, or may be quite inconspicuous. Chromosomes undergoing endomitosis never seem to reach the degree of condensation characteristic of a true metaphase; even at their maximum condensation they remain irregular in outline. In certain types of somatic cells in insects these diffuse endopolyploid chromosomes may be counted without much difficulty. Thus in male grasshoppers with 23 chromosomes in the diploid set the layer of cells which forms the thin sheath of the testis follicles contains nuclei of three different size

classes, with 23, 46 and 92 irregular chromatic masses. Such a tissue is a 'mosaic' of diploid, tetraploid and octaploid cells. It is characteristic of endopolyploid tissues that they are almost always mosaics of cells of different 'ploidies'. Only very rarely do we find tissues with a uniform degree of endopolyploidy.

In certain insect tissues endopolyploidy may be developed to such an extent that the nuclei contain tens of thousands of chromosomes. In such cases they cannot, of course, be actually counted and the degree of 'ploidy' can only be determined by indirect means. In some instances the sex chromosomes (X or Y) can be distinguished because of their evident heteropycnosis. Thus some of the branching salivary gland nuclei of male pond-skaters (*Gerris lateralis*) contain 256 X-chromosomes and are consequently 512-ploid. Two larger size classes of nuclei are presumably 1,024- and 2,048-ploid, but the X's cannot be directly counted in these.

Theoretically it should be possible to determine the degree of ploidy of giant somatic nuclei by measuring their DNA content, but there are considerable technical difficulties in the way of such determinations in the case of very large nuclei.

Although most endopolyploid nuclei have lost the power to divide by mitosis there are some exceptions, one of which is especially significant. In the cells of the ileal epithelium of metamorphosing mosquito pupae endopolyploid nuclei undergo somatic reduction divisions, so that the adult mosquito has ileal cells with 12 or 24 chromosomes ($4n$ or $8n$) instead of the 48 or 96 that were present in the larva.

CHAPTER IV

Polytene and Lampbrush Chromosomes

In a few special types of cells unusually large chromosomes exist which reveal structural details that cannot be seen in ordinary somatic chromosomes. Such giant chromosomes are of enormous importance to the science of cytogenetics. Even if they have not led to a precise answer to the question 'what is a gene?' they have provided invaluable information bearing on the intimate structure of the genetic material.

There are two main kinds of giant chromosomes, the *polytene* type which occur in certain larval tissues of Dipterous flies, especially the salivary gland nuclei, and the *lampbrush* type which occur in the oocyte nuclei of certain vertebrates, particularly the Amphibia. These two sorts of giant chromosomes are entirely different in appearance and seem to originate in quite different ways. Thus far, the polytene chromosomes have been far more extensively used for cytogenetic purposes, but use of the lampbrush elements is on the increase. It has proved to be a most fortunate circumstance, unsuspected in the early days of *Drosophila* genetics, that these insects happen to possess giant polytene chromosomes, which are entirely unknown in other animals and do not even occur in all species of Diptera. The largest lampbrush chromosomes unfortunately occur in slow-breeding animals that are singularly unsuited for genetic work.

Polytene or salivary gland chromosomes

Giant chromosomes of this kind are strictly confined to

certain types of somatic tissues in the insects belonging to the order Diptera (crane-flies, midges, mosquitoes, houseflies, *Drosophila*, etc.). Usually they attain their largest size in the spherical nuclei of the larval salivary gland, but similar nuclei frequently exist in other tissues such as the lining cells of the gut and its derivatives, the malpighian tubules, as well as in the muscle and fat body cells, etc. Thus the general term polytene chromosomes is preferable to salivary gland chromosomes, although in practice it is the nuclei of the salivary gland which are used for routine cytogenetic analysis. The technique used for studying them consists in crushing the glands in a solution of acetocarmine or aceto-orcein, so that the nuclear membranes are ruptured and the chromosomes spread out and flattened. Preparations obtained in this manner do not show the nuclei as they are in life, but they reveal details of the chromosomes that cannot be seen in intact nuclei.

Polytene chromosomes are giant worm-like structures which show transverse bands that stain with ordinary chromosomal dyes, including the Feulgen reagent, so that we may be certain they are rich in DNA. These bands can be shown to run right through the thickness of the chromosome, i.e. they are not merely on its surface. Between the bands are interband regions which are relatively devoid of nucleic acids. The crushing process involved in making a microscopical preparation of polytene nuclei stretches the chromosomal elements and extends the interband segments, so that the dark-staining bands are artificially separated and rendered more easily analysable.

In the case of *Drosophila melanogaster*, the chromosomes of each polytene nucleus, when flattened in this way, appear as five long strands and one quite short one attached to a central mass known as the chromocentre; to which the single large nucleolus is also attached. The

relationship between these six strands and the eight chromosomes of the ordinary mitotic set of this species (Fig. 7) is not at first obvious. The explanation depends on two facts: (1) the two members of each pair of chromosomes are closely fused throughout their length, (2) the centromeres of all the chromosomes together with the heterochromatic segments adjacent to them are all joined together to form the chromocentre. Thus, of the six strands the short one represents the two fused IVth chromosomes, one long one represents the X, while the remaining four are the limbs of the V-shaped chromosomes II and III. In salivary gland nuclei from female larvae the strand representing the X is double like the others, while in nuclei from male individuals it is single, the Y being quite small and almost completely included in the chromocentre. Fusion of strands is not necessarily restricted to twos in polytene nuclei since in triploid *Drosophila* larvae all three homologues usually fuse, so that the number of apparent strands is the same as in diploid nuclei. However in the midge *Lestodiplosis* there is one 'super-giant' cell in each salivary gland which is both polytene and 32-ploid, but in this case pairing is limited to twos.

Chromocentres occur in all species of *Drosophila*, their size depending on whether the proximal heterochromatic segments are extensive or not. In some other groups of Diptera such as the midges of the families Sciaridae and Chironomidae a chromocentre is absent. Thus in species of *Chironomus* with $2n = 8$ there are four separate worm-like elements in the polytene nuclei, each resulting from the fusion of two chromosomes throughout their length. Absence of a chromocentre in these forms does not imply that they have no heterochromatic segments in their chromosomes; in fact we know that they do.

Fusion of homologous regions in polytene nuclei

occurs even in individuals heterozygous for chromosomal rearrangements. Thus if a chromosome pair is heterozygous for an inversion one homologue will usually wrap around the other so as to produce a 'reversed loop'. Individuals heterozygous for deficiencies will show small unpaired loops, while those which are heterozygous for translocations will show cross-shaped configurations in their polytene chromosomes. Thus study of salivary gland preparations under the microscope enables cytogeneticists to detect chromosomal rearrangements with great ease and rapidity. The closeness with which the homologues are paired in the polytene nuclei varies somewhat from species to species and is rather loose in some Chironomidae.

The significance of the polytene chromosomes is much greater than this, however. The individual bands differ greatly among themselves, so that they can be recognized and identified. It is thus possible to construct cytological 'maps' in which the position and characteristic appearance of each band is indicated. By studying *Drosophila* stocks carrying minute deficiencies and other tiny chromosomal rearrangements it has been possible to assign many genetic loci to particular bands.

The polytene chromosomes are not of uniform diameter throughout, but have various 'waists' and other thin places, as well as characteristic enlargements called 'puffs' or 'bulbs' that are constant in position. In addition, there are certain 'puffs' which are specific to certain tissues, or to a particular stage of development in a tissue; we shall discuss the significance of these later.

The generally accepted interpretation of the polytene chromosomes assumes that they may be compared to somatic chromosomes that have become unspiralized and have replicated repeatedly without effective separation of the resulting strands. Some of the bands obviously consist of a layer of dark staining chromomeres

seen from the side; probably all the bands are really of this nature but in many the chromomeres are more or less intimately fused together. The chromomeres of the successive bands must be connected by thin chromonemata, the whole structure being something like a telephone cable. Polyteny is thus seen as a special case of the much more general phenomenon of endopolyploidy. In fact some diptera such as *Miastor metraloas* have endopolyploid salivary nuclei of 'normal' type, and transitional types between 'normal' endopolyploidy and polyteny have been described in various species of Diptera. It is tempting to assume that the existence of polytene chromosomes depends on the 'somatic pairing' force which is generally characteristic of the Diptera and absent or very feebly developed in other groups of organisms. Determinations of the amount of DNA in a fully developed salivary gland nucleus of *Drosophila* and comparison with 'ordinary' somatic nuclei suggests that the largest polytene nuclei contain about a thousand times as much DNA and are hence perhaps 2,048-ploid. In *Chironomus* the degree of polyteny has been estimated to be as high as 16,000 (i.e. these nuclei are perhaps 2^{14}-ploid).

The total number of bands that can be seen in the stretched X-chromosome of *Drosophila melanogaster* is somewhat over 1,000 and the total number in the whole karyotype about 5,100. This is probably a minimum estimate which could be increased by more detailed study. Many of the thicker 'bands' can be shown by stretching to be compound, i.e. composed of several thinner bands with very short interband intervals. There are a great many instances where two bands of identical appearance lie next to one another in the chromosome; these are known as *doublets* or (when their edges tend to fuse) *capsules*.

A study of *Drosophila* stocks with minute deficiencies

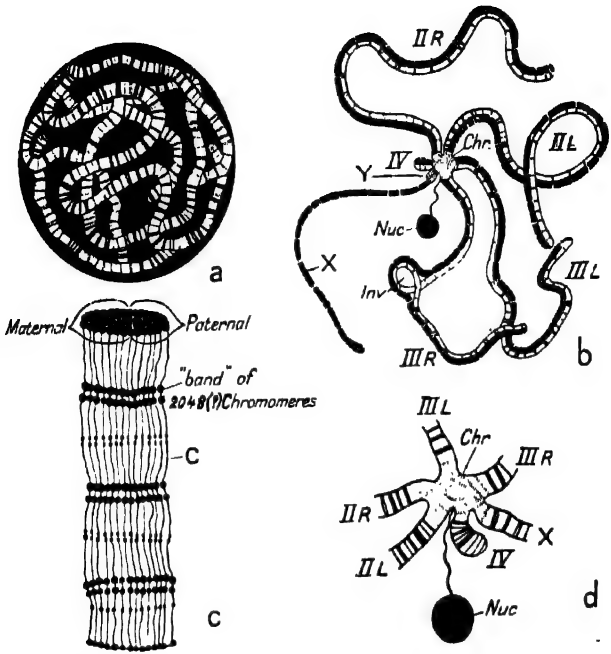


Fig. 7. Diagrams illustrating the structure of polytene chromosomes

a, general view of a salivary gland nucleus with the chromosomes coiled within it. Nuclear sap shown black. *b*, chromosomes of a salivary gland nucleus of a male *Drosophila melanogaster*, spread out by crushing the nucleus. The maternal parts of the paired chromosomes are shown in black, the paternal parts white. *Chr*, chromocentre; *nuc*, nucleolus. II L and II R are the two limbs of the second chromosome, III L and III R those of the third chromosome. The Y is very small. A heterozygous inversion is shown in the III R limb. *c*, diagram of a small part of a polytene 'chromosome' showing the details of the bands, made up of fused chromosomes. *d*, diagram showing details of the chromocentral region.

has enabled cytogeneticists to discover that many genetic loci are confined to particular bands. Thus the classical *white* locus, which is now known to consist of two genes (or subgenes?), is apparently located in the doublet numbered 3C 2–3, the *Notch-facet* locus in band 3C 7, etc. Some deficiencies of up to 50 bands are viable in the heterozygous condition in *Drosophila melanogaster*, and flies lacking one entire fourth chromosome (at least 137 bands) are also viable. But such large deficiencies are lethal in the homozygous state, and it is only a few 1–3 band deficiencies which are homozygous-viable.

There are a number of regions in the polytene chromosomes of *Drosophila melanogaster* where a short sequence of bands appears to be repeated, but in the reverse order, thus: ABCDEFFEDGHI. It seems probable that these *repeats* have arisen in evolution as duplications; it is even possible that the doublets are to be interpreted as single-band repeats.

The same bands are present in the polytene chromosomes of different tissues such as the salivary glands, malpighian tubules, rectal epithelium, etc. But the appearance of some of the bands may be very different, bands that are thin and darkly staining in one type of cell being swollen into diffuse puffed-up structures in another. These differences seem to reflect the functional activity of the individual bands and are hence a very direct expression of cellular differentiation. The general appearance of the polytene chromosomes varies considerably with the nutritional state of the individual and in order to obtain chromosomes suited for detailed investigation it is generally necessary to ensure optimum nutrition and slow development of the larvae. Certain particularly striking puffed-up regions of the polytene chromosomes are known as Balbiani rings. These seem to be genetic loci engaged in very active biosynthesis, the individual threads or chromonemata being all pushed

out from the chromosome in long loops. In the midge *Acricotopus* there are two types of cell in the salivary gland. In one type of cell two Balbiani rings (which we can call A and B) are developed while in the other type there are no Balbiani rings at these loci, but two other Balbiani rings C and D are strongly developed at two other loci. This kind of thing suggests that certain genetic loci are far more active in some tissues than in others, and that the consequences of this are directly visible in the polytene chromosomes.

Much genetic work on *Drosophila* and other organisms has been devoted to the elucidation of so-called *complex loci*. These are 'genes' in the classical sense, within which crossing over can take place between certain sub-loci. Many complex loci exhibit the phenomenon of *pseudoallelism*; two mutants produce a different phenotype according to whether they are in the same chromosome (*cis* configuration) or in opposite chromosomes (*trans* configuration). Symbolically, $\frac{a_1 a_2}{+\ +}$ is more nearly wild type than $\frac{a_1\ +}{+\ a_2}$. Some complex loci appear to be located in salivary gland doublets, but certain of them contain 3, 4 or even 5 sub-loci and it does not seem that each sub-locus is invariably represented by a single visible band in the salivary gland chromosomes.

'Lampbrush chromosomes'

The nuclei of oocytes during the 'growth phase', which corresponds to the middle part of the meiotic prophase, are usually very large. This is especially so in the case of those vertebrates such as sharks, amphibia reptiles and birds, whose ova are large and yolky. In some amphibia, these oocyte nuclei may reach a diameter of half a millimetre. In view of this, and the fact tha the 'DNA values' of the Urodeles are higher than in any other

group of organisms, it is not surprising that we find giant oocyte chromosomes in newts and salamanders. In fact, the longest diplotene chromosomes of such species as *Triturus cristatus* and *T. viridescens* are about 700–800 micra in length at the mid-diplotene stage of meiosis (see p. 74), without any artificial stretching. They are hence by far the longest chromosomes known, even though their diameter is much less than that of the dipteran polytene chromosomes.

These extremely long diplotene chromosomes occur as pairs, held together only at a few points, where they appear to touch. These points must represent chiasmata (see p. 75). Chromosomes of this type may be studied in the unfixed and freshly isolated condition, preferably by phase contrast and with an inverted optical system in which the preparation is viewed from below, so that the chromosomes come to rest on the upper surface of the cover-glass and flatten out under their own weight.

Under such circumstances each chromosome appears to consist of a row of granules or chromomeres approximately 1 μ in diameter but of different sizes and shapes, some approximately spherical, others ovoid or elongated in the long axis of the chromosome. From these chromomeres pairs of loops project out laterally. Typically, each chromomere bears a pair of loops, but in some instances more than one pair may appear to arise from a chromomere. Probably these apparently single chromomeres with more than one pair of loops are compound, resulting from fusion of several independent chromomeres.

The length and appearance of the loops varies greatly, i.e. the successive loops along the length of the chromosome show major differences in size and shape. The loops also go through a series of changes as the growth of the oocyte proceeds. Thus in the early stages the loops are relatively short and the chromomeres large, but as

the nuclei reach their maximum size the loops become much longer, the longest ones extending out as much as 200–300 micra from the chromomeres, which have by now become much smaller. One has the impression that in this process the loops are 'spun out' from the chromomeres, which consequently shrink in size.

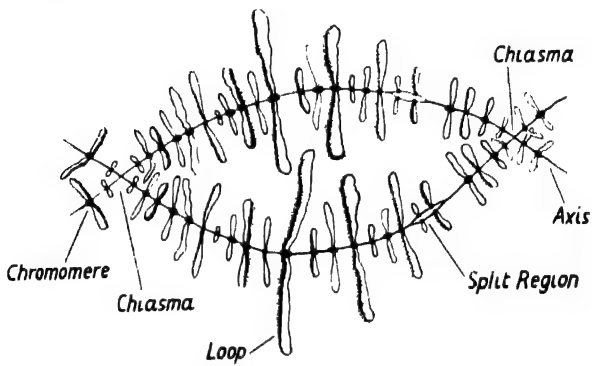


Fig. 8. Diagram of part of a mid-diplotene lampbrush bivalent from an oocyte of the newt Triturus marmoratus *showing chiasmata, axis, chromomeres, lateral loops and a split region.*

The number of chromomeres and loops is actually much greater than that shown in the diagram. Based on the work of Callan and Gall.

Apparently each loop consists of an axis (which is presumably made of DNA since it is resistant to digestion by pepsin and ribonuclease but is destroyed by DNA-ase) surrounded by granular material (protein and RNA) that can be stripped off by dilute saline or proteolytic enzymes. In fact the loops appear to be elaborating materials which are sloughed off from them in the course

of the growth phase; in all probability what we are seeing here (even though not all details are directly visible) is the actual 'functioning of the genes' (as we might have said in the 1930's) or 'transfer of genetic information' (as we are more likely to put it in the 1960's).

There may be 6,000-10,000 chromomeres in the entire chromosome set of *Triturus cristatus*, which agrees very well with the estimated number of bands in the salivary nucleus of *Drosophila*.

Since the chromomeres lie in regular rows there is clearly a connecting thread or chromosome axis between them. But an interchromomeric strand cannot be resolved by the light microscope. It has, however, been detected by electron microscopy and seems to be about 200-400 Å in diameter. By analogy with other diplotene chromosomes both chromomeres and axis should be double – in other words each chromosome should consist of two chromatids. Doubleness is not ordinarily visible, however, even in electron micrographs. However, there are two additional reasons for supposing that a true duality does exist. The first is that in the salamander *Triturus marmoratus* a few short regions do show a double row of chromomeres, spaced apart and each bearing a *single* loop. These are presumably regions in which the chromatids are separated. The second is, of course, that in ordinary regions apparently single chromomeres bear pairs of loops and this certainly suggests that what appears as a single chromomere is really two side by side closely pressed together.

The hundreds of long loops which project out from these amphibian oocyte chromosomes give them a characteristically hairy appearance which has been likened to an old-fashioned brush for cleaning oil lamp chimneys – hence the name of 'lampbrush' chromosomes. At the end of the prophase stage the lampbrush bivalents contract down to much smaller dimensions.

At the same time the loops disappear, being apparently absorbed back into the chromomeres. By the time the first metaphase stage is reached the bivalents are quite small and of normal appearance.

Diplotene and pachytene bivalents in both spermatogenesis and oogenesis of other organisms also have a 'hairy' or 'woolly' outline, but the details are hard to make out. It seems highly probable that lateral loops are universally present at this stage and that the structure of all diplotene chromosomes is fundamentally the same, those of amphibian oocytes being merely larger and easier to interpret. To some extent this may be due simply to the large quantity of DNA per nucleus in the amphibia and especially in the Urodeles.

When lampbrush chromosomes are subjected to stretching the chromomeres first separate as the interchromomeric axis elongates. If stretching is continued some of the chromomeres break transversely through the middle, the broken portions being still connected together through the persistent loops. This suggests that some of the chromomeres, at any rate, may be similar to the 'doublets' in the polytene chromosomes.

The loops appear to have an axis that is composed of DNA, since it is resistant to digestion by proteolytic enzymes and by RNA-ase. But the granular material which covers this axis and makes up most of the thickness of the loop probably consists of RNA and protein since it is removed by these enzymes. Only DNA-ase completely disperses the loops and at the same time leads to fragmentation of the axis of the chromosome between the chromomeres.

The fact that neither proteolytic enzymes nor ribonuclease lead to a break-up of the lampbrush chromosomes, but that DNA-ase does cause a fragmentation between the chromomeres, is the strongest evidence we have for the view that the whole chromatid is a single

DNA molecule several or many centimetres in length, no doubt complexly folded or coiled in the chromomeres and probably also between them and in the loops. A lampbrush chromosome would consist of two chromatids a hundred or two Ångströms in diameter. In the loops these are completely separated from one another, while between successive chromomeres they are so closely paired that even in the electron microscope they appear as a single strand.

Amphibian oocyte nuclei contain in addition to the lampbrush chromosomes large numbers of RNA-protein granules and masses of free nucleolar material which appear to be derived from the loops. It is difficult to escape the conclusion that each loop pair represents a genetic locus, the RNA-protein material cast off from it being a direct product of genic activity. There is probably a close analogy between the loops of the lampbrush chromosomes and the Balbiani rings of the dipteran polytene elements. In both cases we are directly observing the biochemical activity of genetic units, but in the salivary nuclei the biosynthetic processes are largely restricted to the elaboration of vast quantities of a few specific proteins, while in the oocyte nucleus almost all the genetic loci are biochemically active – hence the much greater number of loops in the oocyte nuclei compared with the 1–2 Balbiani rings in the salivary gland nuclei.

Modern work provides no support for the view that the lampbrush chromosomes are polytene and in fact seems to be incompatible with this interpretation.

Certain individuals of *Triturus cristatus* exhibit heterozygosity for specific loops in the oocyte nuclei. A long and conspicuous pair of loops on one homologue may be represented by a pair of much shorter ones on the other homologue which, however, arise from a larger

chromomere. This kind of thing has been interpreted as a direct expression of allelic differences.

Nothing in our knowledge of lampbrush chromosomes seems to exclude the possibility that all the DNA of the chromosome is in the form of a single giant fibre – a supermolecule with a molecular weight of many millions. Such a fibre would itself have a diameter of about 20 Å and would hence be coiled or folded even in the interchromomeric axis (which has a diameter of 200–400 Å), and much more complexly coiled in the chromomeres and in the axes of the loops, which are thicker. It does not seem to be known just how much DNA is present in the haploid nucleus of *Triturus cristatus*. But two other salamanders, *Necturus* and *Amphiuma*, have 24×10^{-12} and 84×10^{-12} grams respectively (see Table I). If we imagine all this DNA as a Watson and Crick double helix, it would amount to about 6·8 metres in one species and 23·8 metres in the other, the average length per chromosome being 57 cm. in one case and 2 metres in the other. It is not known what differences exist between the chemical structure of the DNA strand (*a*) in the interchromomeric axis, (*b*) in the chromomeres, (*c*) in the loops. Neither is it clear why the sections of DNA in the loops seem to be exempt from the intimate pairing that exists throughout the rest of the chromosome (with the exception of the special regions in the chromosomes of *Triturus marmoratus*, previously noted). If it is true (as it appears to be) that in these oocyte nuclei chiasmata are only formed in the intervals between chromomeres and never between one loop and another, this could be very significant in connexion with the problem of defining the gene concept.

CHAPTER V

Meiosis

Meiosis is the antithesis of fertilization. In diploid organisms it results in the chromosomes being reduced to the haploid number. If meiosis takes place immediately after fertilization (as it does in most of the Sporozoa and in the Charices, Basidiomycetes and Ascomycetes among plants as well as in some of the algae) the 'adult' organism will be haploid. If on the other hand meiosis occurs just before fertilization, during gamete formation (as it does in all higher animals) the adult will be diploid. In the higher plants, with an alternation between sporophyte and gametophyte generations, meiosis takes place during spore-formation, i.e. occupies an intermediate position in the life cycle, where the gametophyte generation is the predominant one (as in mosses and liverworts) the 'adult' plant will be haploid; where it is the sporophyte which is predominant (in ferns and higher plants) the 'adult' will be diploid. The same general principles apply in the case of polyploid species.

Meiosis has been defined as two divisions of the nucleus in the course of which the chromosomes only divide once. The whole process must be regarded as having arisen through profound modification of two mitotic divisions. Although aberrant and modified types of meiosis do occur in some species of animals and plants it is really remarkable how constant the details of the normal meiotic process are throughout the great

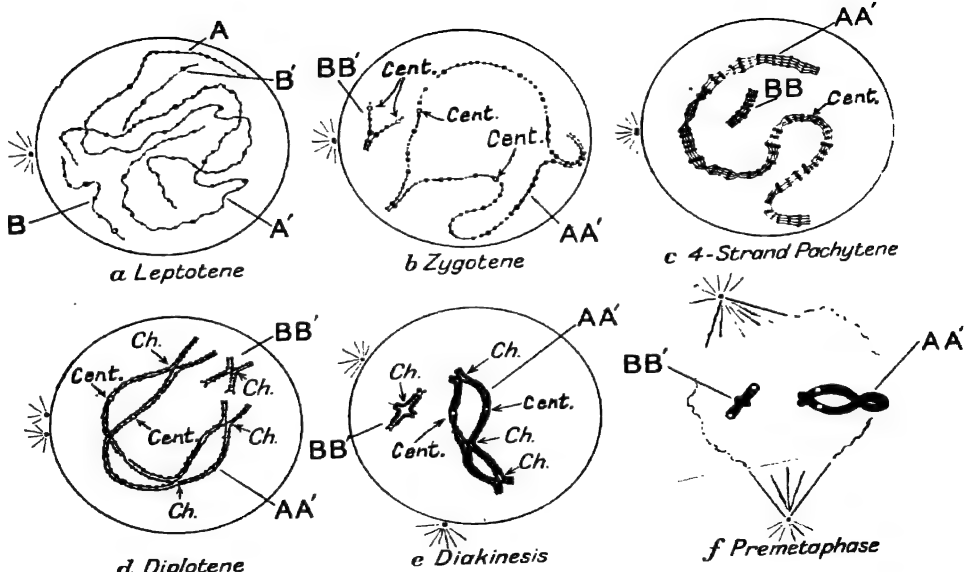
majority of the higher animals and plants. And although the cytoplasmic phenomena of meiosis are quite different in the two sexes, the behaviour of the chromosomes is almost precisely the same in oogenesis and spermatogenesis, in the formation of the pollen grains and in megasporogenesis. A general description of what happens to the chromosomes in the course of meiosis will hence apply to all these diverse cases. In the following account we shall describe meiosis in a diploid individual; the meiosis of polyploids is necessarily more complex and will be dealt with later (p. 104).

The first meiotic division always begins with a lengthy prophase stage; since this differs from a simple mitotic prophase in many respects it is necessary to subdivide it, for purposes of description, into a number of stages which, although they correspond in a general way to the early, mid- and late prophase stages of mitosis, have different names to indicate the appearance of the chromosomes as they undergo various transformations. The names of these stages are, in order, *leptotene*, *zygotene*, *pachytene*, *diplotene* and *diakinesis*. After diakinesis (which corresponds to the end of prophase) comes a short premetaphase, followed by the metaphase of the first meiotic division.

Leptotene

The leptotene stage is usually one in which the chromosomes are very elongated and slender and have a *polarized* orientation with all their ends directed towards one small area on one side of the nucleus. This polarization does not seem to affect the centromeres at all; it is a property of the ends or telomeres, and cannot be said to be a relic of the orientation pattern of the previous telophase.

There has been some controversy as to whether the leptotene strands are optically single or double


A
B'
B
A'
a Leptotene
BB'
Cent.
Cent.
Cent.
AA'
b Zygotene
AA'
BB
Cent.
c 4-Strand Pachytene
Ch.
BB'
Cent.
Ch.
Cent.
Ch.
Ch.
AA'
d Diplotene
AA'
Ch.
Ch.
Cent.
BB
Cent.
Ch.
Ch.
e Diakinesis
BB'
AA'
f Premetaphase

('unsplit' or 'split'), some cytologists having claimed to have seen visibly split leptotene threads, while others have denied that a split can be seen. In the plant *Tradescantia* replication of DNA does not seem to be completed until the zygotene stage, while in *Trillium* DNA synthesis is not completed until pachytene.

Zygotene

Leptotene is usually a rather brief stage. It is followed by the zygotene ('mating thread') stage in which the homologous chromosomes come together in pairs and become closely approximated throughout their length. This process of *pairing* or *synapsis* usually seems to start at the chromosome ends and then proceeds, zipper-like, along the length of each chromosome pair, until it is complete and there are no unpaired regions left, except for some sex chromosomes or other special regions which may be present in the haploid state, so that they have nothing to pair with. It is important to realize that the pairing which occurs at zygotene is a very intimate one which is not merely between homologous chromosomes but always between strictly homologous regions. Thus where some chromosomal segments have become rearranged so that, for example, one chromosome is homologous to parts of two others, all the corresponding regions will be, in general, attracted together and become synapsed. And if one member of a pair of homologues has a segment inverted (by comparison

Fig. 9. Diagrams of the main stages of meiosis

Two pairs of chromosomes AA′ and BB′ are shown, the A and A′ chromosomes having submedian centromeres, the B and B′ elements having subterminal ones (i.e. the B pair are acrocentric or subacrocentric). *Ch*, chiasmata; *Cent.*, centromeres. No terminalization of chiasmata is shown.

with the sequence in the other homologue, so that one may be represented by ABCDE and the other by ADCBE) the two mutually inverted regions will twist around and form a 'reversed loop' in such a manner that each region is in contact with the corresponding region in the other chromosome, gene for gene and no doubt down to the finest subdivision of the chromosome that has any biochemical meaning.

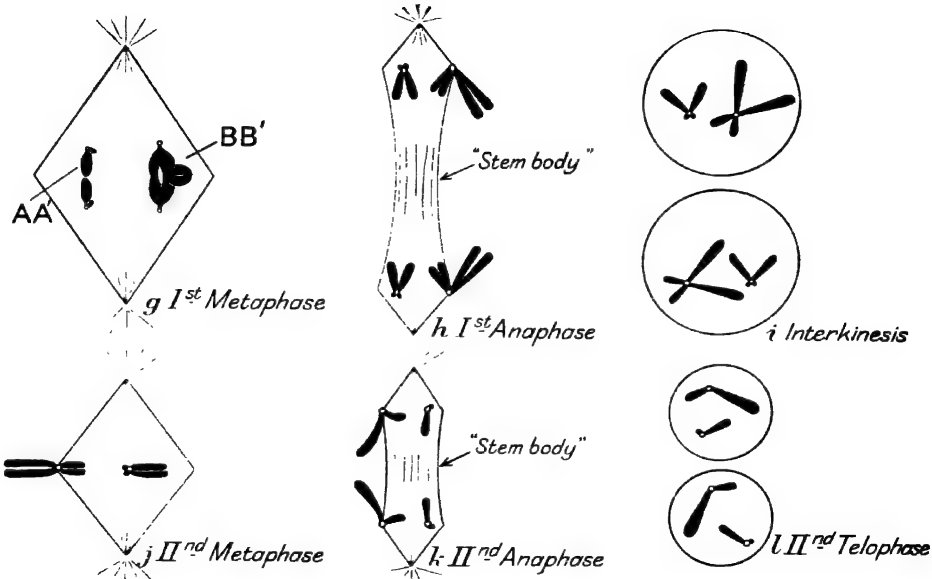
The synaptic process is undoubtedly facilitated by the polarized orientation of the chromosomes, which brings their ends close together. The nature of the force of attraction that is involved is not understood; but it is presumably of the same nature as the 'somatic pairing' force of the Diptera (p. 21). In both cases the fact that all homologous regions can undergo synapsis, even in very complex structural heterozygotes, argues in favour of a force that is operative over distances of several micra.

Pachytene

The pachytene stage may be said to have begun as soon as synapsis is complete. Usually it is a long stage and there may be differences in the appearance of early and late pachytene nuclei. In some species the chromosomes appear quite diffuse, in which case the term 'confused' stage has been applied by some authors. More usually the degree of condensation increases, so that by late pachytene the chromosomes are relatively thick threads, although still with 'woolly' outlines.

As a result of synapsis, the apparent number of chromosomes (in a diploid organism) has been reduced to half; if there were $2n$ chromosomes in leptotene there will be n associations of two chromosomes at the

Fig. 10. Diagrams of the main stages of meiosis (continued from Fig. 9)


AA'
BB'
g I st Metaphase
"Stem body"
h I st Anaphase
i Interkinesis
j II nd Metaphase
"Stem body"
k II nd Anaphase
l II nd Telophase

beginning of pachytene. These associations of pairs of chromosomes are called *bivalents*. Each pachytene bivalent appears, under the light microscope, to be made of two strands between which a split is visible. Thus a pachytene bivalent closely simulates the appearance of an ordinary mitotic chromosome at mid-prophase, although it has arisen in a totally different way, by pairing of two entirely distinct chromosomes, instead of

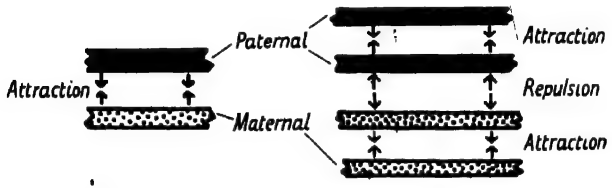


Fig. 11. Diagram illustrating the hypothesis that an attraction between 'unsplit' strands is replaced by a repulsion between 'split' ones as pachytene passes into diplotene, so leading to the 'opening out' of the bivalents between the chiasmata

by splitting of a single one. In very late pachytene the two constituent chromosomes of each bivalent become visibly two-stranded, so that the whole structure is four-stranded. These strands are approximately equidistant from one another so that they form a square when seen in cross-section.

The polarized arrangement of the chromosomes which is so characteristic of leptotene and zygotene usually disappears to a considerable extent during pachytene, although traces of it may still persist in some organisms.

Diplotene

At the end of the pachytene stage the synaptic attraction between the homologues seems to suddenly come to an

end and they would separate completely from one another were it not for the fact that in each bivalent there are certain places where two out of the four strands form an X (see Fig. 12). Each of these places is known as a *chiasma* (plural: *chiasmata*). Some bivalents, particularly the shorter ones, show only a single chiasma, the bivalent looking like a cross (X or +). Other bivalents may have several chiasmata; in that case the bivalent looks like a short length of chain, with loops or links between the successive chiasmata.

As soon as the chiasmata have become visible, due to the lapsing of the synaptic attraction, the diplotene stage may be said to have begun. Chiasmata are now known to be an almost universal feature of the diplotene stage in all higher organisms (a few exceptions will be mentioned later, pp. 100–103). In species that show chiasmata there is always (apart from very rare instances) at least one chiasma in each bivalent – in other words bivalents without a chiasma at all do not occur, except as very rare anomalies, in species whose meiosis involves chiasma formation. Bivalents with 2, 3 and 4 chiasmata are common in many species, but numbers above 4 are rather rare. The highest number of chiasmata that has been counted in a single bivalent is probably 12, in the long chromosome of the broad bean, *Vicia faba*. The average number of chiasmata is known as the chiasma-frequency, and one may speak of the chiasma-frequency of a particular chromosome or of the whole karyotype. The former seems to range from 1·0 to about 8·6 (in the longest chromosome of the domestic chicken). Except for bivalents which invariably show a single chiasma (which occur commonly in many species) the number of chiasmata seems to be inherently variable, i.e. the same bivalent may show 1 chiasma in a particular cell, 2 in another and 3 or 4 in yet another.

It was long ago realized that there were two possible

ways of interpreting chiasmata, and for a long while it was uncertain which was correct, or whether one might be correct in certain instances and the alternative in other cases. The true situation was elucidated largely by the work of JANSSENS and DARLINGTON. On the first or *classical* hypothesis no breakage of the chromatids has taken place before the time of appearance of the chiasmata, and the four threads are consequently unaltered. On this hypothesis a chromatid of paternal origin actually 'crosses over' one of maternal origin (in the literal topological sense), in such a way that on one side of the chiasma a paternal chromatid is paired with a paternal and a maternal with a maternal, while on the other side a paternal is paired with a maternal and a maternal with a paternal.

On the second or *chiasmatype* hypothesis two of the four strands have broken in the pachytene stage and rejoined diagonally in such a way as to produce an X (Fig. 12). On the first hypothesis a chiasma *might* give rise (by subsequent breaking) to a genetic crossover; while on the second hypothesis a genetic crossover (breakage and reciprocal re-fusion) has preceded the appearance of the chiasma and given rise to it. On the chiasmatype hypothesis a paternal chromatid is associated with another paternal one and a maternal with another maternal one on *each* side of the chiasma. It is now generally accepted that the chiasmatype hypothesis is true and the classical hypothesis false. There are a number of topological proofs of the chiasmatype hypothesis; what is probably the simplest one is given in Fig. 12 *e* and *f*. It sometimes happens that there is an unequal pair of homologues, either the maternal or the paternal chromosome being longer than the other. If a single chiasma is formed in the homologous region, the result will be an 'unequal bivalent' as in Fig. 12*e* and not as in *f*. Some even more convincing proofs of the

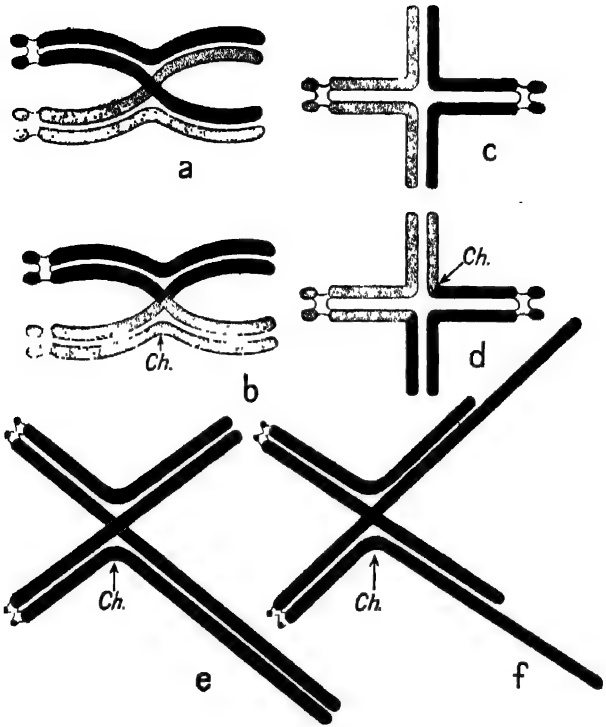


Fig. 12. Diagrams illustrating the difference between the classical and the chiasmatype theory of chiasmata

Maternal chromosome black, paternal one stippled in *a d*. *a*, classical interpretation; *b*, chiasmatype interpretation; *c*, the same bivalent on the classical interpretation after rotation; *d*, the same on the chiasmatype interpretation after rotation. *e* and *f* provide a proof that the chiasmatype theory is correct. *e* is an unequal bivalent with a single chiasma as actually found, *f* is what would happen in such a bivalent if the classical theory were true (never found).

chiasmatype hypothesis depend on observations of meiosis in polypoids or in cells where two bivalents have got accidentally interlocked during synapsis.

Each chiasma is thus a visible sign that a genetic crossover has taken place. We may accordingly estimate the total amount of crossing-over in a particular chromosome or in the chromosome set as a whole simply by counting the chiasmata under the microscope – a fact which is of considerable genetic significance. Acceptance of the chiasmatype hypothesis does not, however, tell us very much about the actual mechanism of crossing-over. The main questions seem to be the nature of the force which causes the chromatids to break, the reason why a paternal and a maternal one always break at the same level, and why all four strands do not break at the same place. Furthermore, it is known that there is a certain minimum distance between successive chiasmata, so that the occurrence of one diminishes the probability of formation of a second one for a certain distance on either side ('*chiasma interference*') – a phenomenon which any satisfactory theory of the mechanism of crossing-over must take into account.

BELLING proposed that crossing-over occurs simultaneously with chromosomal replication, by what we might call a *copy-choice* mechanism. This would imply that the crossover strands were always the new ones, the old strands remaining intact. In this form BELLING'S theory fails to account for bivalents with 2 chiasmata which involve 3 or 4 chromatids ('three-strand double exchanges' and 'four-strand doubles'). Thus to save the theory it was necessary to postulate that in addition to the cytologically and genetically detectable crossovers a large number of sister strand crossovers occurred – which would be undetectable by most genetic methods and presumably invisible cytologically. There is a good deal of evidence that sister strand crossovers either do

not occur or occur too rarely to give essentially random numbers of 2-strand, 3-strand and 4-strand double changes, on BELLING's hypothesis. And the hypothesis appears quite untenable also on the basis of TAYLOR'S analysis of the method of chromosomal replication, according to which each strand is half-old and half-new.

Various hypotheses have been put forward according to which crossing-over results from some localized strain or tension in the bivalent, which is in some way relieved by breakage and re-fusion 'the other way round'. But these hypotheses hardly lend themselves to experimental proof or disproof.

The bivalents in the diplotene nuclei are still elongated and relatively slender. The transition to the next stage, or *diakinesis*, involves a gradual thickening and shortening of the strands. To some extent, also, they lose the hairy or woolly appearance which is characteristic of diplotene. At the same time the successive loops between the chiasmata come to lie in planes which are perpendicular to one another (exactly as in a stretched metal chain). We may regard this orientation of the loops as due to rotation through 90° from the plane of the early diplotene bivalent. Some bivalents with a single chiasma (which we may regard as composed of two incomplete loops) undergo rotation through 180°, so that they end up as flat crosses of the type shown in Fig. 12*c* and *d*.

During the time when the slender diplotene bivalents are gradually becoming changed into the thick, heavy-staining bodies characteristic of the diakinesis stage, there is in some species of animals and plants a tendency for the chiasmata, or some of them, to slip along towards the ends of the chromosomes. This process of *terminalization* is far from universal, but in species which show it the positions of the visible chiasmata, as seen in late diplotene or diakinesis, no longer correspond to the

places where crossing-over took place at pachytene. As a result of terminalization some of the chiasmata may actually reach the ends of the bivalents; but even so the homologues are still held together, as if the chiasma was unable to slip right off the end. DARLINGTON

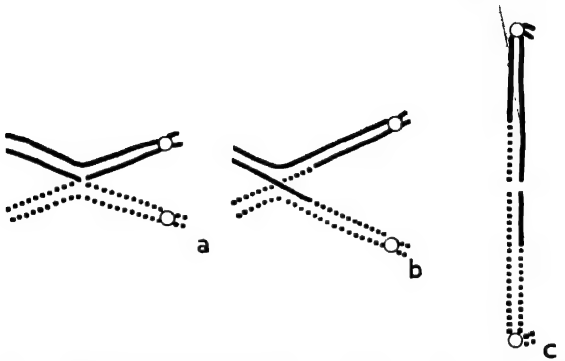


Fig. 13. Diagram showing three stages in the terminalization of a chiasma

Paternal strands black, maternal ones dotted. *a*, diplotene; *b*, diakinesis; *c*, first metaphase.

supposed that a special 'terminal affinity' was responsible for holding the chromosomes together in a terminal chiasma, but it now seems much more likely that the ends of the chromatids are held together by the still undivided telomeres in the manner shown in Fig. 14*a*.

Diakinesis

There is no essential difference between late diplotene and diakinesis, but the bivalents become shorter, thicker and more darkly staining. 'Rotation' is usually completed by the beginning of diakinesis, so that the

loops between the chiasmata are all at right angles to one another, but 'terminalization' may continue right up to first metaphase. Sometimes there is a tendency for

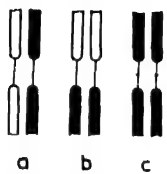


Fig. 14. Diagrams of terminal chiasmata

In *a* and *b* the paternal and maternal chromatids are shown in black and white. Chromatids are connected by visible, dark staining threads which may have an enlargement in the middle (shown in *c*). According to DARLINGTON some terminal chiasmata may be as in *b*, but the author believes them to be all as in *a*.

the bivalents to move out to the inner surface of the nuclear membrane during diakinesis.

Premetaphase

During the premetaphase of the first meiotic division the nuclear membrane disappears and the spindle forms, rather rapidly. At the same time the bivalents become attached to it, the two centromeres of each bivalent coming to lie on opposite sides of the equatorial plane, one 'above' and the other 'below' it. This is a very essential difference between the first meiotic division, and an ordinary, somatic mitosis in which the centromeres become orientated exactly on the equator. The difference does not depend on the fact that the chromosomes are associated as bivalents, but more probably results from the actual state of the centromeres (effectively divided at mitosis, functionally undivided at meiosis). The evidence for this is twofold: (1) unpaired,

i.e. univalent, chromosomes at meiosis frequently attach to the spindle somewhere between the equator and the pole, (2) associations of two chromosomes which have essentially the structure of bivalents can be produced at somatic divisions by radiation-induced translocations, yet they orientate with both centromeres on the equator of the spindle.

In some species of animals the bivalents seem to get violently stretched on the spindle at premetaphase so that they become quite thin and elongated, but this premetaphase stretch is not found at all in many species, and seems to be generally lacking in plants. Premetaphase can usually be distinguished from full metaphase because the spindle is still somewhat indefinite in shape and the bivalents are not all uniformly orientated with their centromeres equidistant from the equatorial plane.

First metaphase

Like other metaphases, that of the first meiotic division is a relatively static stage, so that it is not easy, and frequently impossible, to determine by inspection whether a cell is in 'early' or 'late' first metaphase. The chromosomes have by now reached their maximum degree of condensation, and their outlines appear smooth, as a rule. Nevertheless, it has been possible to show, by special techniques, that the chromonema of each chromatid is helically coiled, the gyres being closely pressed together in the living chromosome so that the spiral structure is not immediately visible. In a few instances it has even been possible to demonstrate a 'minor' spiral as well as a 'major' one, the chromonema having the same kind of structure as the 'coiled coil' filaments of certain electric lamps.

The distance between the two centromeres of each bivalent, at first metaphase, depends on the position of the most proximal chiasma or chiasmata (if there is one

on either side of the centromeres). If these are quite close to the centromeres the latter will, of necessity, lie near one another, only slightly above and below the equator of the spindle. On the other hand, if there is a considerable distance between the chiasmata and the centromeres (either because the chiasmata were originally formed in a fairly distal position or because they have undergone terminalization) the centromeres will be attached to the spindle about midway between the equator and the poles, or even closer to the latter. In a bivalent with several chiasmata, some on one side of the centromere and some on the other, the loop in which the centromeres lie will, as a result of stretching, lie vertical, i.e. in the plane of the spindle axis, at first metaphase. The next loop, on either side, will lie horizontal, the one after that vertical and so on. Free chromosome arms, beyond the most distal chiasma, may be regarded as incomplete loops and will obey the same rules. In some organisms with very condensed chromosomes it may not be possible to count the loops or the chiasmata at first metaphase, and in others all the chiasmata will have completely terminalized by this stage.

First anaphase

It is characteristic of the anaphase of the first meiotic division that the centromeres do not divide. Instead, each whole centromere moves in the direction of the nearest pole, behaving in this way like the daughter centromeres of an ordinary mitotic division. In doing so, the centromeres drag after them the chromatids which are attached to them. This forces the chiasmata along the bivalent until they finally slip off the ends as the half-bivalents are torn asunder and move up the sides of the spindle, which is now actively elongating. Chromosome ends which are held together by 'terminal chiasmata' (i.e. by undivided or terminally fused

telomeres, according to our interpretation) are likewise pulled apart, whatever chemical bonds held them together being broken at this stage.

In the case of metacentric chromosomes the half-bivalents which pass to the poles at first anaphase will be four-armed structures, with the centromere at their point of junction. In the case of acrocentric elements two of the arms of the cross will be very short and probably undetectable at this stage, so that the separating half-bivalents will appear V-shaped.

The result of the first metaphase is often said to be a separation of whole chromosomes instead of split halves of chromosomes, as at mitosis. While this is correct in a sense, it should be pointed out that the chromosomes which separate at first anaphase are not the same, genetically, as the maternal and paternal elements which came together at zygotene; these have interchanged sections of their length by crossing-over, so that the actual chromosomes which separate at the first meiotic division are *new* combinations of segments of paternal and maternal origin. Between the centromere and the first point of crossing-over on either side of it, however, the first anaphase always leads to the separation of two maternal from two paternal chromatids. And between the first crossover and the next one distal to it, the first anaphase invariably involves the separation of a paternal and a maternal strand from a maternal and a paternal. Thus the first meiotic division is 'reductional' between the centromere and the first crossover and 'equational' between the first and second crossovers (what happens distal to the second crossover depends on whether we are dealing with 2-strand, 3-strand or 4-strand double crossing-over).

Whether the maternal or paternal centromere of a particular bivalent goes to a particular pole ('North' or 'South') is a matter of chance, depending on the way the

bivalent has orientated itself at premetaphase. In general, there is no correlation between the mode of orientation of the bivalents in the same cell. Thus in *Drosophila melanogaster* with 4 bivalents all the paternal

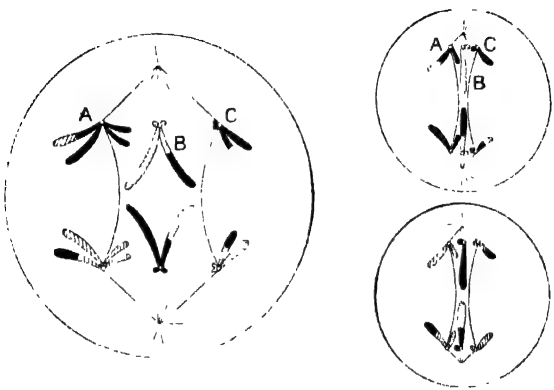


Fig. 15. Diagrams showing the genetic consequences of the first and second meiotic divisions

Maternal portions black, paternal ones cross hatched. Three pairs of chromosomes are shown, each pair having possessed a single chiasma. It will be seen that the first division is always 'reductional' between the centromere and the first chiasma and that the second division is always 'equational' for this region.

centromeres will go to the same pole once in 8 (2^3) times, in grasshoppers with 12 bivalents once in 2,048 (2^{11}) times, and in man with 23 bivalents once in 4,194,304 (2^{22}) times.

Telophase and interkinesis

The telophase of the first meiotic division does not differ essentially from that of an ordinary somatic mitosis.

However, the chromatids of which each chromosome is composed are widely separated, so that each metacentric element is an X-shaped structure and each acrocentric is a V (actually an X with two of the limbs so short as to be invisible). The spindle has by this time elongated considerably, and only remains as a slender 'stem-body' between the two telophase nuclei. Cytokinesis may or may not take place at this stage. In some organisms the two telophase nuclei pass into a more or less complete resting stage (interphase or interkinesis) between the two meiotic divisions, in which the chromosomes become unfixable as in a somatic interphase. But in other cases the interkinesis stage is more or less telescoped out of existence and the telophase nuclei pass directly into the prophase of the second meiotic division, or in extreme cases into the premetaphase stage of that division.

Second meiotic division

The prophase of the second meiotic division, even if present as a distinct stage, is always short and does not include any of the complications which occur in the first meiotic division. The spindles of the second meiotic division become organized rapidly, and the premetaphase stage has been reached. There are at this stage only two noteworthy differences from an ordinary somatic division: (1) the number of chromosomes is half the somatic number, (2) the chromatids diverge widely, being only held together at the centromere and not approximated throughout their length as at mitosis. The major spiral has been lost during interkinesis, so that the spiral of the second meiotic division seems to be derived from the minor one of the first division, by diminution in the number of gyres and increase in their amplitude. Second division chromatids are characteristically rather slender and may show irregular kinks

which are probably remnants of the major spiral of the first division.

At the beginning of second anaphase the centromeres (which, it will be realized, have remained undivided since the last premeiotic division) divide and begin to move towards the poles (previously they have been situated on the equator as in an ordinary somatic division). Since the chromatids are already quite free from one another, there is no mechanical obstacle to rapid anaphase separation. Genetically, the second division is necessarily 'equational' for all those regions for which the first was 'reductional' and vice versa. Thus the second division is always equational at the centromere and from the centromere to the position of the most proximal chiasma.

The telophase of the second meiotic division does not differ from that of a somatic division, except for the number of chromosomes.

In particular organisms meiosis may be arrested at various stages, until some stimulus causes the resumption of the sequence of changes. Thus in the sexually immature individuals of many species spermatocytes in the pachytene stage accumulate and do not proceed into diplotene until a certain stage of development has been reached. And in many insect eggs (strictly speaking, oocytes) the nucleus becomes 'blocked' in the premetaphase or metaphase of the first meiotic division and does not develop further until the sperm has penetrated.

CHAPTER VI

Chromosomal Rearrangements

It is probable that the changes which we call gene mutations or point mutations are alterations in the sequence of nucleotide pairs in the DNA molecules. Such changes are on a molecular level, quite invisible even with the electron microscope. Structural changes of an altogether different order of magnitude occur, however, in chromosomes, from time to time, in which the sequence of regions is altered. MULLER (1956) has argued most eloquently that these should be regarded as quite different phenomena.

The frequency of occurrence of structural changes in chromosomes can be greatly increased by ionizing radiation and to some extent also by various drugs. 'Spontaneous' structural changes also occur from time to time in the chromosomes. Many of these are of types which are unsuited to survive. However, there is evidence that some of them do survive since a very large fraction of the visible differences between the chromosome sets (karyotypes) of related species have arisen by chromosomal rearrangement (Ch. XI) and the natural populations of many animals and plants are polymorphic for changes of this kind.

Chromosomal rearrangements arise as a result of two kinds of events, *breakage* and *reunion*. All chromosomal breaks, however caused, seem to be essentially similar, and presumably fracture the same kind of chemical bonds. They lead to the formation of freshly broken

ends which differ from the natural chromosome ends (telomeres) in being 'sticky', i.e. liable to undergo reunion. Telomeres are not 'sticky' and will not unite either with other telomeres or with freshly broken ends. There is no polarity about freshly broken ends, i.e. they cannot be regarded as positive or negative and any freshly broken end will join up with any other such. Once a reunion has occurred the junction is as firm as before, so that the chemical bonds are probably of the same kind as the original ones.

Any chromosome break may undergo *restitution*, i.e. it may reunite in such a way as to restore the original sequence. If this happens we cannot say that a rearrangement has occurred.

The main types of structural chromosomal changes, as seen at metaphase, in monocentric chromosomes, may be classified as follows:

(1) *Single chromosome breaks* (terminal deletions). Both chromatids are broken at the same level. There will be a proximal *centric fragment* and a distal *acentric fragment*. Such single breaks may and frequently do undergo restitution. Alternatively the 'sticky ends' of the two acentric chromatids may fuse to give a U-shaped fragment ('distal sister strand union') and the corresponding ends of the centric chromatids may do likewise ('proximal sister strand union'). Sister strand union occurs rather regularly in the case of breaks produced by mechanical stretching of the chromosomes on the spindle at anaphase-telophase, but less frequently after breaks induced at certain mitotic stages by radiation. Acentric fragments will be lost in the cytoplasm regardless of whether they have undergone sister strand union or not. Centric fragments which have undergone sister strand union give rise to dicentric chromatids at the next mitosis which are usually mechanically broken as they are stretched like a bridge on the spindle when the

two centromeres pass to opposite poles. Since these mechanical breaks may be followed once more by sister strand union, single chromosome breaks may initiate a theoretically endless series of breakage-fusion-bridge cycles, the distribution of the genetic material to the daughter cells becoming progressively more divergent from the normal, until loss of the chromosome or death of the cells containing it occurs.

For these reasons single chromosome breaks cannot, in general, give rise to permanently viable cytogenetic changes. Many of the deleterious effects of ionizing radiations, at the cellular level, may be ascribed to single chromosome breaks leading to loss of acentric fragments and to breakage-fusion-bridge cycles. There is evidence from radiation experiments that chromosome breaks are of two kinds: (*a*) due to breakage *before* DNA replication, a '*chromatid break*' being replaced by a *chromosome* break, i.e. one through both chromatids at the same level, (*b*) due to breakage of both strands *after* replication, at the same level ('isochromatid breaks' of CATCHESIDE and LEA).

(2) *Chromatid breaks*, in which only one chromatid is broken, the other remaining intact.

(3) *Inversions*. These result from two breaks in the same chromosome with rotation of the segment between them through 180° and fusion of the sticky ends. A chromosome with the sequence of regions ABCDEF, if broken between B and C and between D and E, becomes ABDCEF after the inversion. Inversions may originate from either chromatid or chromosome breaks. We may distinguish between *paracentric* inversions (where both breaks are on the same side of the centromere) and *pericentric* ones (where the two breaks are on opposite sides of the centromere).

(4) *Deletions*. Two breaks occur in the same chromosome limb (i.e. on the same side of the centromere) and

the segment between them is lost, as an *acentric fragment*, while the remaining sticky ends join up. Frequently the two ends of the acentric fragment may join together so as to form an acentric *ring fragment*, but this is lost in the cytoplasm in any case.

(5) *Ring chromosome formation.* Two breaks occur in the same chromosome, on opposite sides of the centromere; the ends of the centric fragment fuse, to form a *centric ring chromosome*, leaving the two acentric terminal fragments to their fate (it is immaterial whether they fuse together or undergo sister strand reunion – being acentric they will degenerate in the cytoplasm anyhow). We shall consider the behaviour of ring chromosomes at mitosis and meiosis later.

(6) *Mutual translocations* (interchanges). Two breaks occur in different, non-homologous chromosomes or chromatids. In either case, there are two ways in which reunion may occur, (*a*) in such a manner as to produce two *monocentric* chromosomes, each containing parts of the two original ones, (*b*) so as to give rise to a dicentric and an acentric chromosome or chromatid. Some authors refer to (*a*) as *symmetrical* interchanges, (*b*) as *asymmetrical* ones. The latter are obviously incapable of survival in organisms with monocentric chromosomes.

(7) *Duplications or repeats.* These may be *tandem* or *reversed.* A tandem duplication may be represented by the sequence ABCDCDEF, a reversed one by ABCDDCEF. The former may result from mutual translocations between homologous chromosomes, or by a similar type of rearrangement involving the two chromatids of a single chromosome. The latter probably arise mainly through a fusion between an acentric fragment and a chromatid mechanically broken in an anaphase bridge following sister strand union.

More complex types of rearrangements may result

from three or more chromosome or chromatid breaks in the same nucleus or from various combinations of chromosome and chromatid breaks. Three breaks in the same chromosome may lead to tandem inversions or to the region between breaks 1 and 2 being *inserted* into break 3 (either inverted or non-inverted). Four breaks in the same chromosome may lead to *independent*, *included* or *overlapping* inversions.

A special type of reversed duplication is where we have a metacentric chromosome with the two limbs homologous throughout (e.g. *xyz*C*zyx*, where C represents the centromere). Such isochromosomes, as they have been called, may arise in various ways, following on breaks through or adjacent to the centromere. Attached-X chromosomes in *Drosophila melanogaster* are isochromosomes which have been extensively studied in genetic experiments.

Mitotic and meiotic behaviour of ring chromosomes

Ring chromosomes have been studied especially in maize and *Drosophila melanogaster*. In the latter, ring-X chromosomes have arisen on a number of occasions, but no ring-shaped autosomes are known, and are hence probably inviable or unstable.

Most ring chromosomes seem to suffer from certain mechanical disabilities at mitosis, which lead to their destruction. The most common type of accident is a kind of sister strand crossing-over. A single such crossover in a chromosome consisting of two parallel ring chromatids converts it into a single double-length dicentric ring chromatid. At the following anaphase the two centromeres will pass to opposite poles, stretching a double chromatid between them. In maize, these bridges are broken under tension, and the freshly broken ends then proceed to re-join in the two daughter nuclei. Thus in the course of a few division cycles a great

variety of ring chromosomes of different sizes, some containing extensive deletions and others with large duplications, are produced. This kind of breakage-fusion-bridge cycle will lead eventually to inviable or degenerating tissues. It is thus not surprising that ring chromosomes do not occur in the karyotypes of wild species of animals and plants. Certain types of ring chromosomes are also liable to produce two interlocked ring chromatids at anaphase.

The ring-X chromosomes of *Drosophila melanogaster* are apparently rather more stable than most other ring chromosomes, so that laboratory stocks carrying such ring-X's can be maintained. In females with one ring-X and one normal acrocentric X, crossing-over is almost completely normal, so that a bivalent can apparently be formed between them, and crossing-over is not prevented, although certain types of single chiasmata and certain types of doubles will give rise to anaphase bridges which are left in the polar nuclei and do not get into the egg nucleus.

'Whole arm rearrangements'

Translocations in which the breakage points are adjacent to the centromeres need to be considered as somewhat different from ones in which the breaks are some distance from the centromere. The former leave the chromosome arms virtually intact and consequently lead to 'whole arm rearrangements'. Three types are especially important: (1) a change whereby two metacentrics AB and CD exchange limbs so that two new chromosomes AC and BD are formed. At meiosis in the heterozygote a ring of four chromosomes is formed and if this orientates itself with the alternate centromeres directed towards the same pole (zig-zag configuration) the gametes will receive a complete set of genes; (2) a change in which one acrocentric chromosome

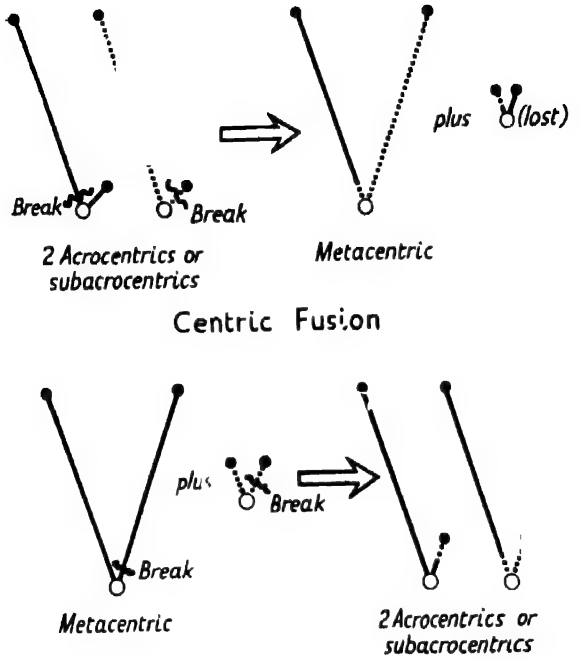


Fig. 16. Diagram showing the main ways whereby chromosome numbers undergo decreases and increases

In *centric fusion* two acrocentric or subacrocentric chromosomes undergo a translocation to give a large metacentric together with a very small metacentric, which is lost. In *dissociation* a large metacentric and a small supernumerary chromosome fragment undergo a translocation which results in a metacentric.

Centromeres represented by hollow circles, telomeres by small black circles, positions of breaks indicated by wavy lines.

breaks immediately to the 'right' of the centromere, the other just to the 'left' of it (i.e. one breaks in the long arm, the other in the minute short arm). This leads to the formation of a large metacentric and a very small one which is likely to be lost in the course of the next few generations if (as will usually be the case) it consists mainly of heterochromatin that is relatively inert, genetically. This kind of translocation is called a *centric fusion* or simply a fusion; (3) the opposite kind of change to a centric fusion is where we start out with a large metacentric and a minute fragment chromosome and end up with two acrocentric chromosomes. Such a change may be called a *dissociation*.

Centric fusions and dissociations have been important in the chromosomal evolution of animal and (probably to a lesser extent) plant species. Apart from reduplication of whole chromosomes in polyploidy they represent the main way in which evolutionary decreases and increases of chromosome number have come about (see Ch. XI). Certain other types of whole arm rearrangements (e.g. tandem fusions, whereby two acrocentrics produce a double length acrocentric and interchanges whereby a metacentric AB and an acrocentric C give rise to a metacentric AC and an acrocentric B) are theoretically possible and can be studied in genetic experiments, but are unlikely to be successful in the evolution of natural populations and species because they are liable to lead to a severe reduction in the fertility of the heterozygotes.

CHAPTER VII

Special Problems of Meiosis

We have seen in Chapter V that at least one crossover normally occurs in each pair of chromosomes at meiosis. If a bivalent has a chiasma frequency of 1·0, that is equivalent to saying that it invariably forms a single chiasma (since bivalents with no chiasmata do not normally occur). A chiasma frequency of 1·0 also means that two out of the four chromatids are single crossover strands, i.e. that between two genetic loci situated at opposite ends of the chromosome crossing-over takes place in 50% of cases. Thus a bivalent with a chiasma frequency of 1·0 will have a total map length of 50 genetic units; similarly bivalents with chiasma frequencies of 2·0 and 3·48 will have map lengths of 100 and 174 units, respectively – we simply multiply the chiasma frequency by 50 to get the map length.

The above relationship is derived theoretically, from the chiasmatype theory. It is interesting to see how actual data support it. The total chiasma frequency for the entire karyotype of the maize plant is about 27·05, which would indicate a total map length of 1,353 units. If we add up the genetic maps of the ten chromosomes we obtain 904 units. The discrepancy is not unexpected, since it is unlikely that genetic markers are known at or even very close to all of the chromosome ends (if the length as calculated from genetic data had been *longer* than that predicted from cytological data we might have had reason to doubt the theory).

In some chromosomes it appears that chiasmata are formed with equal frequency in all segments of equal

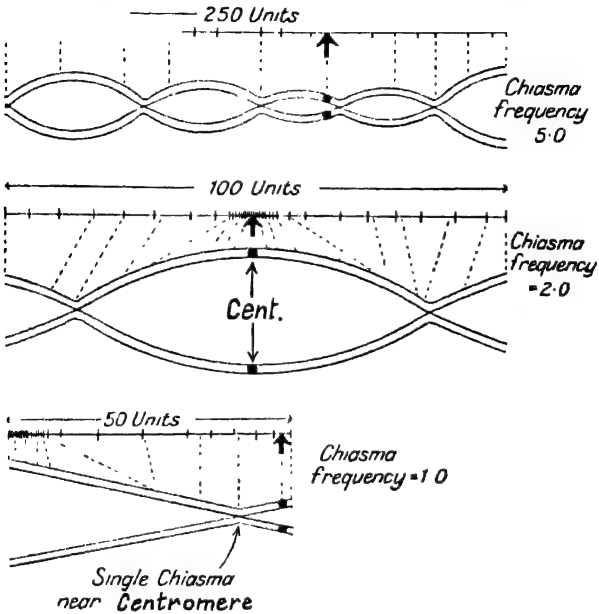


Fig. 17. Diagram showing the relation between chiasma formation and genetic maps

Regions where chiasmata are seldom or never formed will show an apparent crowding of genetic loci. Further explanation in text.

cytological length. In such cases we may say that the chiasmata are not localized in any way. This appears from the published data to be approximately true in

the case of the long chromosomes of *Vicia faba*, *Lilium* spp., the grasshoppers *Stenobothrus* and *Chorthippus* and the newt *Triturus cristatus*. However, even in these cases it may well be that chiasmata have a greater chance of occurring in certain regions than in others of the same length. In very many species of animals and plants large deviations from a random distribution of chiasmata are found, and in extreme cases very strict localization occurs, the chiasmata being more or less rigorously confined to certain regions of the chromosome, other regions having a zero or near-zero chiasma frequency. Some chromosomes have *proximal localization* (the chiasmata being mainly or entirely confined to the neighbourhood of the centromere) while others have *distal* localization. A combination of the two is not uncommon, chiasmata being present near the tips of the chromosome and near the centromere, but absent in a large intermediate region. Chiasma localization of the proximal type has been studied especially in the grasshoppers *Stethophyma* and *Bryodema* and among plants in certain species of the genus *Fritillaria*. Distal localization is evident in certain other species of grasshoppers such as *Paratylotropidia* spp. and *Austroicetes interioris*. Chiasmata that occur very close to the ends of the chromosomes are, of course, almost completely ineffective, genetically. The female silkworm, in which it has been stated that crossing-over does not occur, is probably an example of an organism with extreme distal localization of chiasmata.

In organisms with long chromosomes one occasionally finds two bivalents interlocked; but this is an extremely uncommon type of meiotic accident. Obviously, it results from a chromosome becoming 'trapped' between two synapsing homologues at zygotene.

Genetically determined abnormalities of meiosis

A fairly large number of gene mutations are now known which cause meiotic abnormalities of various kinds, such as asynapsis (failure of zygotene pairing) or desynapsis (breakdown of pairing at a later stage). A recessive mutation *sticky* in maize causes, when homozygous, many mitotic aberrations and at meiosis the chromosomes adhere together and many become broken at anaphase. A third chromosome recessive in *Drosophila melanogaster* prevents crossing-over and leads to much non-disjunction in the female, although it does not affect the male meiosis. In *Drosophila pseudoobscura* and some related species males carrying certain X-chromosome inversions produce over 90% daughters, regardless of the genotype of the mother. The effect of the inversions (or, more probably, of certain genes associated with them) is to cause the X to divide in both meiotic divisions and the Y to degenerate at the second division. Thus, as a rule, all four spermatids receive an X-chromatid. The few sons produced by such fathers are XO in constitution, i.e. they arise from sperms carrying neither an X nor a Y.

The whole complex mechanism of meiosis is undoubtedly a delicately poised one, so that it is liable to be upset by many factors. It may be profoundly disturbed in hybrids (see p. 107) and may become mildly abnormal as a result of continued inbreeding in normally outbred species.

Isochromosomes, i.e. chromosomes with two homologous limbs (the sequence of regions being ABC·CBA, where the dot represents the centromere), will undergo auto-synapsis at meiosis and crossing-over may occur between their two limbs. This type of crossing-over has been studied in the attached-X chromosomes of *Drosophila melanogaster*, and chiasmata between the

two limbs of isochromosomes have been observed cytologically in other types of material.

Anomalous types of meiosis

In various groups of insects the process of meiosis is more or less profoundly modified from the typical pattern described in Chapter VI. Some of the modifications are superficial and probably do not entail any genetic consequences, while others are more profound and may involve alterations in the genetic mechanism.

In various species of mantids and roaches there is no true diplotene or diakinesis stage in the male and the homologous chromosomes remain closely synapsed until premetaphase, when they begin to be stripped apart at the centromere. Chiasmata are thus never visible as such in these species. Since other members of these groups show typical chiasmata, and since intermediate conditions exist, it seems improbable that genetic crossing-over is actually suppressed. If this interpretation is correct, the only difference between the '*Callimantis*-type' of meiosis and the typical process would be in the suppression of the usual diplotene opening-out of the loops between chiasmata, and there would be no difference in the genetic mechanism as such.

In the 'higher' Diptera such as *Musca*, *Calliphora*, *Lucilia*, *Drosophila* and many others, chiasmata are usually not visible in spermatogenesis, and the chromatids lie closely parallel until the beginning of first metaphase. In *Drosophila* spp. it is well known that there is no genetic crossing-over in the male sex, so that in this case the suppression of chiasma-formation is apparently genuine. However. it has been reported that 'chiasmata' may sometimes be seen in autosomal bivalents of male *Drosophila*. The pairing between the four chromatids of such bivalents is rather loose and it seems fairly clear that occasional exchanges of partner

– or apparent exchanges – do not represent real chiasmata.

Chiasmata are also apparently absent in the males of certain scorpions belonging to the genera *Tityus* and *Isometrus*; we have no way of knowing whether genetic crossing-over is suppressed or not, and the situation in the females is unknown.

Some of the 'lower' Diptera such as the Simuliidae and the Mycetophilidae (fungus midges) have a type of meiosis which is just like that of *Drosophila*, while others such as the mosquitoes and Chironomidae have a typical chiasmate type of spermatogenesis. In the Dipterous families Sciaridae, Cecidomyidae (gall midges) and in one subfamily of Chironomidae, highly aberrant chromosome cycles have been evolved, which involve bizarre types of meiosis, either in the male or in the female, or in both. Thus in the male somatic set of *Sciara coprophila* there are five pairs of chromosomes (including one pair of large metacentrics). There is no synapsis at zygotene and all ten elements are univalents. The first meiotic spindle is a unipolar cone-shaped structure to which *both* the large metacentrics and one member of each of the other pairs are attached. The remaining chromosomes move away from the base of the cone; there is genetic evidence that the four chromosomes which move away are paternal in origin. The first meiotic division thus separates a group of six chromosomes from a group of four; the latter degenerate in a small mass of cytoplasm which becomes cut off from the main cell like a polar body, while the group of six proceed to the second meiotic division. Here a regular bipolar spindle is formed and five of the six chromosomes divide normally, but one of the acrocentrics divides in such a manner that both its halves go to the same pole. The group of chromosomes at this pole form the sperm nucleus, while the other group

degenerates. Only one sperm is accordingly formed from each primary spermatocyte, and it contains more than the haploid number of chromosomes. A complex mechanism involving the elimination of certain chromosomes and their degeneration in the egg cytoplasm occurs during the early cleavage divisions. Meiosis in the female *Sciara* is, however, entirely normal.

In the Cecidomyids (gall midges) there are many chromosomes present in spermatogonial and oogonial nuclei which are not present in the soma (because they have been eliminated during certain of the cleavage mitoses). For example, in *Trishormomyia helianthi* there are twenty-four chromosomes in the spermatogonia, six in the somatic nuclei of the male and eight in those of the female. The first meiotic division of the male leads to the separation of a group of four chromosomes from the remaining twenty, the spindle being cone-shaped, as in *Sciara*. The cell which receives the four chromosomes goes through a second division, which is a simple mitosis, while the other cell degenerates. Thus two sperms are formed from each primary spermatocyte and they contain only four chromosomes each. The female meiosis (of which not all the details have been studied) leads to the formation of an egg nucleus with twenty chromosomes. The elimination processes must operate somewhat differently in male and female embryos, to give the somatic numbers characteristic of the two sexes. Similar chromosome cycles, although with differences in the details, seem to exist in all species of gall midges.

Some highly unusual types of meiosis have been described in some of the Flagellates symbiotic in the gut of the wood roach *Cryptocercus*. In several genera there is a type of one-division meiosis in which no duplication of chromosomes or centromeres occurs, no chiasmata are formed and the homologues segregate

intact to the poles. It has been claimed that these processes represent an intermediate stage in the evolutionary origin of meiosis and sexuality but it seems more likely that they should be interpreted as having evolved from the more usual type of two-division meiosis which is known in many Protozoa.

Divisions at which reduction from the diploid to the haploid number takes place occur in a few insects at stages other than gametogenesis. Thus, in the scale insect *Pseudaulacaspis pentagona* zygotes which are cytoplasmically male eliminate the genetically paternal chromosome set and so become haploid individuals. In another scale insect *Icerya purchasi* there are hermaphroditic individuals with a diploid ovary and a haploid testis which arises from cells in which some kind of reduction division has taken place during the embryonic period. And in lice (order Anoplura) the reduction division in the testis precedes the spermatogonial divisions, which are haploid; in this case there is a single 'meiotic' division which occurs between the last spermatogonial mitosis and the beginning of spermateleosis. It does not involve any reduction in chromosome number, but the cytoplasmic division is very unequal so that large and small cells are produced; the small ones degenerate and only the large cells form sperms. Whether we should call the 'reductional' divisions of *Pseudaulacaspis*, *Icerya* and the Anoplura types of *meiosis* is doubtful: it is unlikely that the usual sequence of meiotic stages (leptotene – first metaphase) occurs in any of them. Another dubious semantic point is whether we should refer to the spermatocytic divisions of organisms with male haploidy as *meiotic*. From an evolutionary standpoint they have clearly arisen from the meiotic divisions of diploid forms: yet they necessarily omit synapsis and chiasma formation and no numerical reduction occurs.

The meiosis of organisms whose chromosomes have multiple or diffuse centromeres deviates in certain respects from the usual pattern. In most such organisms, the chiasma frequency is low – often a single chiasma per bivalent which terminalizes completely. The first division may then be either 'reductional' or 'equational' at the end where the chiasma is (obviously if it is reductional at one end it must be equational at the other, and vice versa). Such types of meiosis occur in some Heteroptera and Homoptera and perhaps in certain other groups.

Meiosis in polyploids

Where there are more than two homologous chromosomes of each kind at leptotene three or more may become synapsed at zygotene. But apparently never more than two chromosomes synapse at any one point. Thus in a triploid, if we consider three homologues A_1, A_2 and A_3, A_1 may synapse with A_2 in one region and with A_3 in another, but A_1, A_2 and A_3 are never associated together in the same region. On the other hand, A_1 may pair with A_2 throughout its entire length (to form a bivalent), in which case A_3 will be left unpaired and form a univalent. Thus in a triploid individual *trivalents*, bivalents and univalents may be found in the same nucleus. Similarly, in a tetraploid, *quadrivalents* may be found in addition to the other three types, and in higher polyploids *quinquevalents* and *hexavalents* may also occur. All associations of more than two chromosomes may be spoken of collectively as *multivalents*. The frequency of multivalent formation in polyploids varies a great deal. Where the chromosomes are very short it may be quite low, almost all the associations in a tetraploid, for example, being bivalents. One reason for this is probably that synapsis can travel very rapidly from one end to the other of

such very short elements. But it is also true that chromosomes which have an inherently low chiasma frequency (i.e. elements which on account of strong interference are incapable or almost incapable of forming more than a single chiasma) will rarely or never be able to exist as multivalents after pachytene – any multivalent associations which they form at zygotene will not be retained into diplotene and later stages.

Another factor of major importance in determining the frequency of multivalent formation is whether the particular polyploid is an auto- or an allo-polyploid; allopolyploids usually form far fewer multivalents than autopolyploids. Thus if we consider an allotetraploid with four chromosomes A, A, *a*, *a*, it will probably form two bivalents AA and *aa*, since although all the chromosomes are partly homologous, those derived from different ancestral species are less completely so than those which are from the same species. But, for the reasons given above, it is not safe to conclude that a polyploid which forms only bivalents is necessarily an allopolyploid.

The general history of multivalents at meiosis follows the usual course already described in the case of bivalents. When they come to orientate themselves on the spindle of the first meiotic division, it is usual in the case of trivalents for two centromeres to be on one side of the equator and the one between them on the other. There are two main types of quadrivalents, *rings* and *chains*. Where the four chromosomes are arranged in a ring it is usual for two centromeres to be orientated towards each pole, but in some instances it is the adjacent centromeres which go together and in other cases the alternate ones. In the case of chain quadrivalents orientation is apt to be less regular and sometimes three centromeres are orientated towards one pole and one towards the other, a type of accident

which occasionally occurs even with ring quadrivalents. In general, where only bivalents and quadrivalents are present, meiosis will be regular, with an equal number of chromosomes passing into each cell. Where univalents, trivalents or multivalents higher than quadrivalents are present meiosis will be mostly irregular,

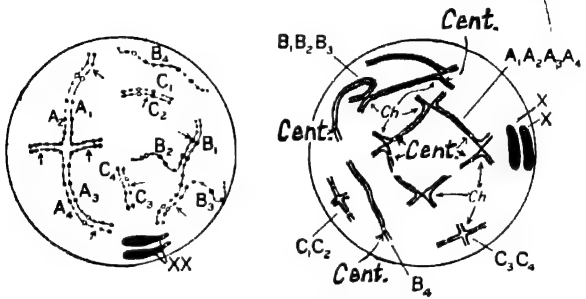


Fig. 18. Diagrams of meiosis in a tetraploid spermatocyte of a grasshopper

Three autosomes A, B and C are shown, together with the X. Thus there are altogether 14 chromosomes. One quadrivalent ($A_1A_2A_3A_4$), one trivalent ($B_1B_2B_3$), two bivalents (C_1C_2 and C_3C_4) and a univalent have been formed among the autosomes. The two X's are held together by a synaptic attraction, but do not form any chiasmata on account of their strong positive heteropycnosis. In *a* the positions where the chiasmata will subsequently arise are indicated by arrows. *Cent.*, centromeres; *Ch*, chiasmata.

with unequal numbers of chromosomes passing to the poles.

Univalents may divide in either the first or the second division, but not in both; frequently they become attached by their centromeres to the equator of the first meiotic spindle and divide tardily or become 'blocked' in the middle of the anaphase spindle.

In XO species of grasshoppers which have X-chromosomes that are strongly heteropycnotic in the male meiosis, but not in the female, the two X's form a bivalent in oogenesis, but when two of them are present in a spermatocyte that has accidentally become tetraploid (in consequence of a failure of one of the spermatogonial divisions) they manifest their homology by lying alongside one another during the prophase of the first meiotic division, but seem to be prevented by their heteropycnosis from undergoing true synapsis and chiasma formation.

Meiosis in species hybrids

In a diploid F_1 hybrid between two different species the two haploid sets of chromosomes will be only partly homologous to one another. If the two parent species had the same chromosome number there may be sufficient homology between the members of the two sets to permit or induce them to pair at zygotene, and the bivalents formed may appear fairly normal in later stages, often with a somewhat reduced chiasma frequency. But equality in respect of chromosome number is not a major factor in determining the degree of pairing. Thus synapsis is often partially inhibited or even absent altogether in hybrids between species having the same chromosome number. Conversely, even where the chromosome numbers differ considerably, the meiosis of the hybrids can be surprisingly normal, two chromosomes of the species with the higher number often pairing with a single one from the species with the lower number to form a bivalent.

The existence of several or many inversions in the heterozygous condition does not necessarily prevent effective synapsis in *Drosophila*, and on the whole there is little evidence that structural heterozygosity of this simple type is an important cause of the asynapsis

observed in species hybrids. Evidence of a similar kind comes from instances where there is extreme asynapsis in hybrids of one sex while synapsis is almost complete in the other sex. This seems to be the case in hybrids between the European salamanders *Triturus cristatus* and *T. marmoratus*, where pairing is very incomplete in spermatogenesis but apparently almost complete in oogenesis.

Most asynapsis in species hybrids seems to be due to some kind of undefined lack of homology; it may be caused in part by slightly different rates or degrees of condensation in the two partially homologous chromosome sets. Even if synapsis and chiasma formation occur normally in a species hybrid, however, that is not a guarantee of fertility, since various aberrations of meiosis may occur at a later stage, due to abnormal development of the spindle or to the relations of the chromosomes to it. Sometimes, as in the male *Triturus* hybrids referred to earlier, there is a mass degeneration of secondary spermatocytes, so that no normal sperms are formed.

The meiotic behaviour in reciprocal hybrids is sometimes strikingly different. Thus in male hybrids from the cross *Drosophila pseudoobscura* ♂ × *D. persimilis* ♀ there is little or no synapsis and the anaphase and telophase of the first meiotic division are highly abnormal. There is no second meiotic division and only giant nonfunctional spermatids are produced. In male hybrids from the reciprocal mating *persimilis* ♂ × *pseudoobscura* ♀ a variable amount of synapsis occurs and the spindles of the first meiotic division undergo an extraordinary elongation, so that they become bent around into a horse-shoe shape.

In many interspecific hybrids such as those between *Drosophila melanogaster* and *D. simulans*, as well as in the male mule, sterility results from the fact that the

spermatogonia fail to develop into spermatocytes, rather than from any abnormalities of synapsis.

The details of spermatogenesis and oogenesis in hybrids are, however, inherently variable, so that chromosomal behaviour may differ from one individual hybrid to another.

CHAPTER VIII

Sex Chromosomes

Where the sexes are combined in a single individual, as in hermaphroditic animals such as earthworms and land snails and in the majority of higher plant species, we do not find any special sex chromosomes in the karyotype. The formation of male and female germ cells in such organisms is accomplished by a process of histological differentiation, either in a hermaphroditic gonad or in separate ovaries and testes.

On the other hand, in bisexual (dioecious) species genetic sex-determining mechanisms are very generally present. A cytoplasmic mechanism of sex determination (i.e. a system where two kinds of eggs are produced which differ cytoplasmically, one kind being destined to develop into males, the other into females) has been reported in the marine worm *Dinophilus* and may exist also in some scale insects. Even where genetic sex-determining mechanisms are present environmental influences may also play a part and may even be preponderant, as in the worm *Bonellia.*

The existence of a genetic sex-determining mechanism does not necessarily imply that cytologically distinguishable sex chromosomes are present. The most widespread type of sex-determining mechanism consists in one sex being heterozygous for certain genetic loci for which the other sex is homozygous. Such loci may be confined to a very short region of one chromosome pair, in which case the sex chromosomes may be re-

garded as composed of a long *homologous region* and a very short *differential region*. Sex chromosomes of this kind are not usually distinguishable from one another, cytologically. They may be regarded as 'primitive' sex

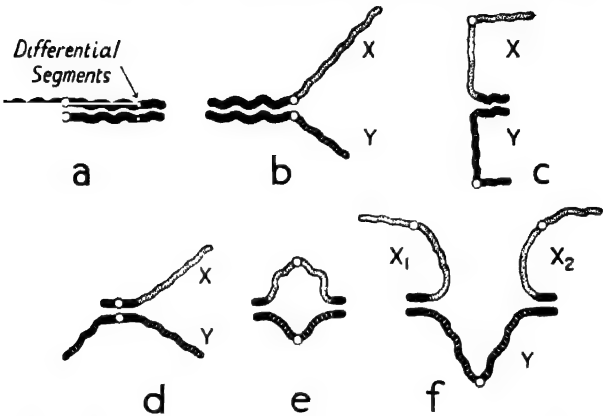


Fig. 19. Diagrams of sex chromosomes, showing pairing segments (black) and differential segments (stippled in the case of the X, *cross-barred in the case of the* Y)

a, where the differential segments are very minute and interstitial (Chironomidae, probably most fishes and amphibia). *b*, where one arm forms the pairing segment, the other being the differential segment (some mammals). *c*, where the pairing segments are distal. *d*, where they are proximal. *e*, where there are two distal pairing segments (many beetles). *f*, an X_1X_2Y system with distal pairing segments (many Praying Mantids).

chromosomes. In more 'advanced' types of sex chromosomes the differential segments have become relatively much longer, at the expense of the homologous regions, which are reduced in length and may even have vanished altogether in some taxonomic groups. Quite

'primitive' sex chromosomes exist, however, in some morphologically 'advanced' groups of organisms. Again and again in the course of evolution one type of sex-determining mechanism has replaced another, frequently more 'advanced' one. The sex which possesses the unequal ('XY') pair of sex chromosomes is called the *heterogametic sex* since it produces two kinds of gametes or spores; the other sex is called the *homogametic sex*. In most groups of animals and plants it is the male which is the heterogametic sex (i.e. there are two kinds of sperms or pollen grains and only one kind of egg or megaspore), but in some groups it is the female which is heterogametic – that is to say there are two kinds of eggs or megaspores, all the sperms or pollen grains being alike (Table III). Some authors refer to XY : XX mechanisms as ZW : ZZ in the case of female heterogamety, but this terminology is not strictly necessary. In the Bryophyta (mosses and liverworts) where the sexual stage of the life cycle is haploid, the male and female gametophytes each contain one member of a pair of sex chromosomes and the sporophyte is XY in constitution.

Differential segments of 'advanced' types of sex chromosomes are frequently heterochromatic. Thus in the genus *Drosophila* Y-chromosomes are entirely heterochromatic, but the X has a long euchromatic differential segment (in some species two differential segments separated by a homologous segment which corresponds to a similar segment in the Y). Chromosomes other than sex chromosomes are referred to as *autosomes*. They are usually euchromatic throughout most of their length, but in some species autosomes which are largely or entirely heterochromatic occur, and could easily be mistaken for sex chromosomes.

In a number of species of animals and in some whole families and orders the Y seems to have been lost from

the karyotype in the course of evolution, so that the somatic number is odd in one sex (usually the male). Such sex-determining mechanisms are usually referred to as XO (male) : XX (female), where 'O' simply indicates the absence of a Y-chromosome. There are even a few species of *Drosophila* in which the males are XO. It is natural to suppose that in these species the Y has been actually lost. More probably, the major part of it has become translocated to the X or an autosome. If so, the genetic (as opposed to the cytological) constitution of these species would be XY (male) : XYXY (female) or XYY (male) : XXYY (female). In the Orthopteroid orders of insects (roaches, mantids, phasmids, grasshoppers, etc.) where XO mechanisms are of general occurrence, the X-chromosomes are entirely heterochromatic.

The sex chromosomes are in most cases (perhaps in all) not the only ones bearing sex-determining genes. Many autosomes probably carry genes which are concerned with the development of characters of one or the other sex; all that the sex chromosomes do is to serve as a mechanism which switches the development of the embryo over to maleness or femaleness at a certain stage. In vertebrates, with circulating sex hormones in the blood stream, the genetic sex constitution can be partially or completely overridden by a change in the balance of the sex hormones. It is thus possible to obtain individuals which are genetically female but phenotypically male. If hormonal sex reversal is incomplete it leads to intersexuality. In insects, which lack circulating sex hormones, parasitization by other insects or by nematodes may lead to a similar intersexuality. These phenomena have nothing to do with the chromosomal mechanism of sex determination as such – they are superimposed upon it and reverse its action, to a greater or lesser extent.

In *Drosophila melanogaster* the X-chromosome is female-determining, the autosomes male-determining (probably especially chromosome 3). Two X's are epistatic over a diploid set of autosomes, so that a female is produced, while one X is hypostatic to two sets of autosomes, thus leading to the production of a male. In *Drosophila melanogaster*, individuals with three sets of autosomes and two X's are intersexual. In this species the Y appears to play no direct role in the determination of the external sex phenotype, since XO males (i.e. ones lacking a Y) are males and XXY or XXYY flies are female. The Y does affect the processes involved in sperm formation, however, so that XO males do not form normal sperm and are consequently sterile.

In some other groups, however, the Y may be actively sex determining. Thus in the axolotl and the silkworm (both of which have XY females) the Y is very strongly female determining, so that almost any individual carrying one or more Y's is female, regardless of how many autosomes it is carrying (in the axolotl the X and Y cannot be distinguished cytologically, so that the interpretation is inferred from genetic experiments). In *Drosophila* YY individuals lacking an X are inviable, and this is probably true in the case of all 'advanced' sex chromosome mechanisms; but in the axolotl it has been shown that YY individuals are viable and female.

In the human species it has recently been shown that the Y plays a major role in determining maleness. Thus XO individuals, which have only 45 chromosomes, exhibit a type of intersexuality known as Turner's Syndrome (Ovarian dysgenesis). Another type of intersexuality, Klinefelter's Syndrome, is apparently due to an XXY constitution (47 chromosomes). Thus evidence as to the male-determining role of the Y comes from both these abnormal conditions. In the mouse, also,

there is evidence that the Y is male-determining, so this may be the usual situation in mammals.

In the Reptiles it is still quite uncertain which sex is heterogametic, since there is no evidence for an XY pair in the males of any of the large number of species that have been investigated and critical cytological studies have likewise failed to reveal a sex chromosome pair in females of several species of lizards and turtles. Sex reversal experiments, such as were employed to investigate the sex-determining mechanism in the axolotl and some other amphibia, have not been tried in reptiles.

In birds there is now abundant cytological evidence for the existence in many species of an X- (or Z-) chromosome which is present once only in the diploid set of females, twice in that of males. The well-known sex-linked loci in the domestic chicken are undoubtedly carried in this chromosome. It is still quite uncertain whether birds possess a Y- (or W-) chromosome in the female; if so it must be one of the smaller elements. At present it seems more probable that they do not, and that the genetic mechanism of sex determination is probably of the *Drosophila* type, but with the roles of the X and autosomes reversed.

Where we have a 'primitive' type of sex-determining mechanism it may be somewhat variable as between different races of a species. Thus the little Central American fish *Xiphophorus* (*Platypoecilus*) *maculatus* includes some geographic races that are heterogametic in the male and others that have female heterogamety. Apparently there are the following types of sex chromosomes in this species:

W – strongly female-determining
X – female-determining
Y – male-determining
Y′ – strongly male-determining

Three races have the following constitution:

	Male	*Female*
Race 1	XY	XX
Race 2	XY′	XX
Race 3	YY	WY

The four types of sex chromosomes in this species are all indistinguishable cytologically, so that the interpretation is derived from genetic experiments in which individuals of different races have been crossed.

Some species of animals and plants have several different kinds of X- or Y-chromosomes which are simultaneously present in the same individual. Numerous kinds of such complex or multiple sex chromosome mechanisms have been recorded. Designating different X's as $X_1, X_2 \ldots$, and Y's as $Y_1, Y_2 \ldots$, the following are the main kinds of mechanisms known:

(*a*) *Multiple X's, single Y*

	Male	*Female*
(*a*1)	X_1X_2Y	$X_1X_1X_2X_2$
(*a*2)	$X_1X_2X_3Y$	$X_1X_1X_2X_2X_3X_3$
(*a*3)	$X_1X_2X_3X_4Y$	$X_1X_1X_2X_2X_3X_3X_4X_4$
	etc.	

(*b*) *Multiple X's, no Y*

(*b*1)	X_1X_2O	$X_1X_1X_2X_2$
(*b*2)	$X_1X_2X_3O$	$X_1X_1X_2X_2X_3X_3$
	etc.	

(*c*) *Single X, multiple Y*

(*c*1)	XY_1Y_2	XX
(*c*2)	$XY_1Y_2Y_3$	XX
	etc.	

(*d*) ***Multiple X, multiple Y***

varying degrees of complexity up to:

$$\left.\begin{matrix} X_1X_2X_3X_4X_5X_6X_7X_8X_9 \\ X_{10}X_{11}X_{12} \\ Y_1Y_2Y_3Y_4Y_5Y_6 \end{matrix}\right\} : \left\{\begin{matrix} X_1X_1X_2X_2X_3X_3X_4X_4X_5X_5 \\ X_6X_6X_7X_7X_8X_8X_9X_9 \\ X_{10}X_{10}X_{11}X_{11}X_{12}X_{12} \end{matrix}\right.$$

In a system with multiple X's there seems to be no genetic homology, or only a partial homology, between X_1, X_2, X_3 etc., so that it would be incorrect, for example, to refer to an X_1X_2Y system as an XXY one,

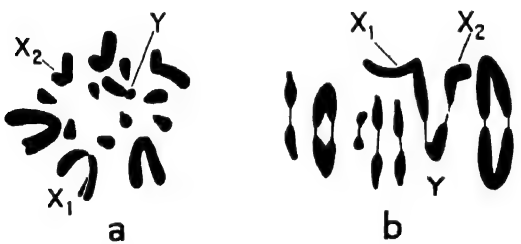


Fig. 20. a, *spermatogonial metaphase, and* b, *first metaphase in an Australian grasshopper with an* X_1X_2Y *sex chromosome mechanism*

The pairing segments in the sex chromosomes are small and distal. This sex chromosome mechanism arose by the method shown in figure 24.

because that would imply that the two X's were homologous. We shall consider the meiotic behaviour of these complex sex chromosome mechanisms later (p. 121).

Sex chromosomes are definitely known in the following species of dioecious plants: *Cannabis sativa* (♂ XY), *Humulus* spp. (♂ XY, $X_1X_2Y_1Y_2$ and XY_1Y_2), *Rumex* subgenus *acetosella* (♂ XY, XXXY, XXXXXY,

XXXXXXXY in diploid, tetraploid, hexaploid and octaploid species), *Rumex* section *acetosa* (♂ XY_1Y_2 and XXXY) and *Melandrium* spp. (♂ XY). A number of other species of dioecious plants have been reported as having morphologically distinguishable sex chromosomes but the evidence is considered insufficient by WESTERGAARD. There can be no doubt that in many species of dioecious plants the difference between X and Y elements is not cytologically detectable. In *Fragaria* the female sex is definitely heterogametic. In *Thalictrum*, *Asparagus* and *Mercurialis*, with male heterogamety, it is apparently the presence of a Y which produces maleness, while in *Rumex acetosa* the Y's are inactive as far as sex determination is concerned and sex depends on the ratio of X-chromosomes to autosomes as in *Drosophila*. In *Melandrium* very extensive investigations, including studies of diploid and polyploid individuals with entire and fragmented Y-chromosomes, have shown that the Y contains a 'female suppressing' region and at least two sections containing genes essential for maleness. The X-chromosome and certain autosomes of *Melandrium* are, to a degree, female-determining.

In two species of microtine rodents, *Ellobius lutescens* and *Microtus oregoni*, the diploid number in *both* sexes is an uneven one, namely 17 (8 pairs of autosomes and a 17th body which is presumably a sex chromosome). It has been suggested that the sex chromosome is an attached XX element in the female and an attached XY in the male, the system functioning as a balanced lethal mechanism, the zygotes which receive 0 or 2 sex chromosomes being non-viable, and only those with a single one surviving.

In a number of species of 'lower' Diptera belonging to the families Chironomidae and Simuliidae the X and Y are alike in shape and in banding pattern (in the polytene chromosomes) except that they differ from one

another in respect of one or more inversions. The male sex is heterogametic in all these species. In the diploid race of the Simuliid *Cnephia mutata* all males, but no females, are heterozygous for a complex rearrangement in chromosome 1. A somewhat more complex situation exists in *Chironomus tentans* in which either chromosome 1 or chromosome 2 can function as a Y or as an X, so that we have $1_{(x)}$, $1_{(y)}$, $2_{(x)}$ and $2_{(y)}$, the two Y-chromosomes differing from the corresponding X's by inversions. Apparently any individual which receives either $1_{(y)}$ or $2_{(y)}$ develops as a male. The switch genes determining maleness are apparently situated in or very close to these inversions.

Behaviour of sex chromosomes at meiosis

The functioning of XY sex chromosome systems depends on these two bodies passing regularly to opposite poles of the spindle at either the first or the second meiotic division, in the heterogametic sex, so that equal numbers of gametes with an X and a Y are produced. In the case of XO mechanisms the principle is the same except that half the gametes will lack a sex chromosome altogether.

In the great majority of mammalian species there is an XY condition in the male. Each element has a homologous region and a differential region (see p. 111) and the two homologous regions appear to synapse at zygotene while the differential regions remain unpaired. One or more chiasmata (in most species probably only one) are presumably formed between the homologous segments although the details are usually obscured by a large nucleolus which is attached to the homologous segment and some authors have doubted whether a chiasma is formed. Thus an XY bivalent attaches itself to the spindle at the premetaphase of the first meiotic division. The centromeres seem to lie very close to the point of junction of the homologous and

the differential segments. Thus when they pass to opposite poles at first anaphase the X-differential chromatids pass to one pole and the Y-differential chromatids to the other. This is called *pre-reduction* of the sex chromosomes. The opposite condition is seen in the fieldmice of the genus *Apodemus* (all except one species) where a chiasma is regularly formed between the centromeres and the differential segments, so that an X and a Y differential segment pass to each pole at the first anaphase (attached to the same centromere) and do not separate until the second anaphase (post-reduction). Post-reduction of X- and Y-chromosomes occurs in many bugs of the order Heteroptera and in a few species of beetles.

The behaviour of XY bivalents in most species of *Drosophila* is essentially as described above for mammals, with pre-reduction universal. It was formerly claimed that a pair of reciprocal chiasmata were regularly formed in the sex bivalents of *Drosophila*, between the two homologous segments, but there is no really critical evidence for this view and the weight of the evidence is against it. There is hence no valid reason for supposing that the association of the X and Y homologous segments in *Drosophila* depends on a different principle to that which holds the autosomes together in the male (it is generally agreed that there are no true autosomal chiasmata in Drosophila spermatogenesis). In several species of *Drosophila* (including *D. melanogaster*) the Y has two differential segments, one on each side of the homologous section; and in some the X has a similar structure, as a result of X-autosome fusions that have occurred in evolution.

In a great many XY species of beetles the Y is much smaller than the X; the two elements seem to have terminal homologous sections with a differential segment in between. Such XY pairs give rise to character-

istic 'parachute-shaped' ring bivalents at the first meiotic division, there being presumably a terminalized chiasma in each of the homologous regions.

In the case of XO mechanisms the X is, of course, a univalent at meiosis. Usually it passes undivided to one pole at the first anaphase (pre-reduction) and divides at the second division; but in a few species of Heteroptera and beetles its behaviour is reversed (post-reduction).

There are some XY mechanisms in such insect orders as the Heteroptera and Neuroptera where no true pairing between the two sex chromosomes seems to occur and where the two sex chromosomes may lack homologous segments. In the Neuroptera the X and Y regularly become orientated on opposite sides of the equator of the first meiotic spindle and pass to opposite poles at anaphase. In many XY Heteroptera the two sex chromosomes are entirely separate at the first meiotic division and divide equationally. Thus all second spermatocytes contain both an X-chromatid and a Y-chromatid. These come together briefly at the second meiotic division and then separate to opposite poles ('touch-and-go pairing'). The mechanism underlying these types of behaviour of sex chromosomes in Neuroptera and Heteroptera remains mysterious, like that which controls the orientation of the sex chromosomes in *Gryllotalpa hexadactyla* and *Eneoptera surinamensis* (see p. 122). In none of these instances do we have any detailed knowledge of the precise spatial relationship of the sex chromosomes to one another during the meiotic prophase.

In species with compound sex chromosome mechanisms of the X_1X_2Y type it is of course necessary that X_1 and X_2 should pass to one pole and the Y to the opposite pole at the first meiotic division (and similarly in the case of XY_1Y_2 systems). In those grasshoppers

and mantids which have compound sex chromosome mechanisms this is apparently ensured by the Y having two pairing segments, one homologous to a section of X_1, the other to a section of X_2. Thus a trivalent is formed, with the Y in the middle, and the alternate centromeres are directed towards opposite poles of the first meiotic spindle, so that disjunction is very regular. In the Hemiptera with compound sex chromosome mechanisms, on the other hand, there is no true meiotic synapsis between the sex chromosomes, any more than there is in most of the species with simple XY mechanisms. Nevertheless in such species as *Acholla multispinosa*, with $X_1X_2X_3X_4X_5Y$ in the male, all the X's go to the same pole at the second anaphase, after a brief 'touch-and-go' approach to the Y.

Even more difficult to understand is the mechanism underlying the functioning of X_1X_2O or $X_1X_2X_3O$ mechanisms – i.e. systems where there are several non-homologous X's in the heterogametic sex, but no Y. This kind of system is almost universal in spiders and is also known in some Nematodes and in a few insects. In these cases the X_1, X_2, X_3 . . . lie parallel and close together and all attach to the same part of the first meiotic spindle, so that they pass together to the same pole at anaphase, thereby ensuring the success of the mechanism.

In males of the mole cricket *Gryllotalpa hexadactyla* there is an X-chromosome which clearly corresponds to the X of related species together with an unequal sized pair of chromosomes which we may designate *xy*, the smaller element (*y*) being confined to the male sex. At the first meiotic division *x* and *y* form a bivalent and are presumably united by a chiasma but X is entirely separate. Nevertheless the *xy* bivalent always orientates itself on the spindle in such a manner that X and *x* pass to the same pole at anaphase, producing

two kinds of sperms, those with X and *x* and those containing only the small *y*.

The cricket *Eneoptera surinamensis* has an X_1X_2Y mechanism, but apparently meiotic segregation takes place without any chiasmata being formed between the X's and the Y.

Gynandromorphs

In certain groups of invertebrates, which lack circulating sex hormones in the blood stream, various kinds of cytological accidents may lead to the production of individuals some of whose tissues and organs are male in type while the remainder are female. Such hermaphroditic individuals, in which there is a sharp boundary between the tissues of the two sexes, are known as *gynandromorphs*. A 'bilateral' gynandromorph is one that has one side of the body male, the other female. There are also antroposterior gynandromorphs, in which the front end of the body is of one sex while the posterior part is of the other. Many 'irregular gynandromorphs' have also been described, in which the body is composed of variously arranged patches of male and female tissue. But the boundary between the male and female regions is always sharp and distinct in gynandromorphs, whereas in *intersexes* all parts of the body combine the characteristics of both sexes and show a condition intermediate between maleness and femaleness. In most species of insects gynandromorphism probably occurs spontaneously in about one out of every 10,000–30,000 individuals, but it can be produced much more frequently by various experimental agencies, such as ionizing radiation, which increase the likelihood of 'cytological accidents' in early development.

Presumably, gynandromorphism occurs in other groups of animals, including vertebrates. But where circulating sex hormones are present it tends to be

masked by their effects; thus a gynandromorphic bird or mammal, in which the male part of the body was supplying male hormones to the cytologically female part, and vice versa, would probably be indistinguishable from a true intersex. Some supposed bird gynandromorphs have in fact been described; they were regarded as such because they were more asymmetrical than most intersexes.

Gynandromorphs have naturally attracted attention in the case of animals in which sex dimorphism is very pronounced, for example in species of butterflies and moths where the wings of the males and females are quite differently coloured or in midges where the antennae are very different in the two sexes. In groups where the secondary sex characters are less developed many cases of gynandromorphism probably pass unnoticed.

The cytological accidents which can give rise to gynandromorphism are of several different kinds. In *Drosophila* gynandromorphs the female tissues are XX, the male ones XO (and not XY). Apparently what happens is that in a genetically female embryo a daughter X-chromosome occasionally gets lost at one of the early cleavage divisions, thereby giving rise to a cell which contains only one X. In some other groups gynandromorphs may arise from binucleate eggs. In the Hymenoptera, with male haploidy, gynandromorphs have their female parts diploid, male regions haploid.

TABLE III

Examples of multiple sex chromosome mechanisms

Male	
*a*1. X_1X_2Y	Many praying mantids (genera *Mantis*, *Tenodera*, *Paratenodera*, *Sphodromantis*, *Hierodula*, *Orthodera*, etc.)
	Grasshoppers *Paratylotropidia brunneri* and *P. morsei* (mid-western states of U.S.A.). Some Eumastacid grasshoppers of Australian subfamily Morabinae (about 11 out of 160 species). The S. American cricket *Eneoptera surinamensis*
	Drosophila miranda
	A few species of fleas
*a*2. $X_1X_2X_3Y$	Reduviid *Sinea diadema*
	Earwig *Prolabia arachidis*
*a*3. $X_1X_2X_3X_4Y$	Water-bugs *Nepa cinerea* and *Ranatra linearis*
*b*1. X_1X_2O	Most species of spiders
	Some Ascarid nematodes
	Some stoneflies of the genus *Perla*
*b*2. $X_1X_2X_3O$	A few species of spiders
*c*1. XY_1Y_2	*Drosophila americana* subsp. *texana*
	Sorrel dock *Rumex acetosa*
	Marsupials *Potorous tridactylus* and *Wallabia bicolor*
	Rodent *Gerbillus gerbillus*
	Shrew *Sorex araneus*

TABLE IV

Groups with male and female heterogamety

Male heterogamety	*Female heterogamety*
Plants	
Gingko	
Rumex (sections Acetosa and Acetosella)	*Fragaria*
Melandrium	
Humulus	
Cannabis	
Thalictrum	
Asparagus	
Mercurialis	
Animals	
Schistosomum spp.	Lepidoptera
Nematodes	Trichoptera
Echinoderms	Copepoda (some, at any rate)
Spiders	Some fishes
Opilionids	Some amphibia
Some mites (others have haploid males)	Birds
Ostracods	
Centipedes	
All insects except the Lepidoptera and Trichoptera and certain groups with haploid males (see Ch. IX)	
Some fishes and amphibia	
Mammals (including 'Echidna')	

Uncertain which sex heterogametic

Brachiopods
Scorpions
Cephalopods
Reptiles

CHAPTER IX

Cytology of Parthenogenesis

In this chapter we shall describe briefly the behaviour of the chromosomes in naturally occurring parthenogenetic reproduction. In animals this involves the development of the egg without fertilization. In the higher plants with an alternation of sporophytic and gametophytic generations a variety of reproductive methods exist which are grouped under the general term *apomixis* and are genetically equivalent to parthenogenesis or (strictly speaking) to certain types of parthenogenesis met with in animals.

Many different kinds of parthenogenesis exist, whose genetic consequences are rather diverse. They have arisen repeatedly, in the course of evolution, from sexual methods of reproduction, with many elaborations of detail. Nevertheless, the inherent genetic limitations of parthenogenetic systems seem to have prevented them from being a long-term evolutionary success in any group. Thus, with the possible exception of the Bdelloid Rotifers there is no family, order or other 'higher category' of animals or plants all the members of which reproduce strictly by parthenogenetic or apomictic methods.

Parthenogenesis and apomixis have developed to a very uneven extent in different groups of organisms. Thus apomixis is frequent in grasses and Compositae, rare or unknown in Gymnosperms, orchids and Leguminosae. In animals it seems to be distributed

sporadically in most insect orders, but is unknown in the Heteroptera and Odonata (dragon flies). There are quite a number of instances known in Crustacea but none in Arachnida except in mites, where many species are known or suspected to be parthenogenetic. In vertebrates only one certain instance is known, in a fish (see p. 133). Parthenogenesis can, however, be induced artificially in almost any biological material, so that the absence of normally parthenogenetic species from certain groups is unexplained.

We shall recognize four main types of parthenogenesis, (1) *haplodiploidy* (or *arrhenotoky*), (2) *automictic* or *meiotic thelytoky*, (3) *apomictic* or *ameiotic thelytoky*, (4) *cyclical parthenogenesis*.

Haplodiploidy

In certain groups of invertebrate animals the males regularly arise from unfertilized eggs, the females from fertilized ones. Males are consequently *impaternate* (i.e. they have no fathers) and 'haploid' (actually, it is only the cells of the germ-line and of a few somatic tissues which are haploid; because of endopolyploidy most of the somatic cells are polyploid in both sexes). This is the state of affairs in all the Hymenoptera (sawflies, ichneumons, wasps, ants, bees, etc.) except for a few that have become secondarily thelytokous. Essentially the same mechanism exists in some scale insects, a few white flies (Aleurodidae), in one species of beetle (*Micromalthus debilis*) and in certain, but not all, species of mites. The males in all these cases lack a true meiosis, only a single mitosis intervening between the spermatogonia and the spermatid stage. Thus the sperm nuclei contain a haploid set of chromosomes identical with those carried by the spermatogonia. In the females meiosis is normal. The genetic mechanism varies somewhat in these haplodiploid groups. In some instances

femaleness depends on heterozygosity for special sex factors; thus in the parasitic wasp *Habrobracon* it is possible to obtain 'diploid' (biparental) males by inbreeding. These exceptional individuals, which are homozygous for the sex factors, produce diploid sperms but are generally sterile. In some other Hymenoptera inbreeding does not lead to the production of biparental males, so that we may conclude that in these instances haploidy *per se* is male-determining, diploidy being female-determining. In the honey bee diploid zygotes homozygous for sex factors of the *Habrobracon* type are apparently inviable, but by the use of marker genes and artificial insemination it has been possible to obtain haploid males with patches of 'diploid male' tissue.

In arrhenotokous parthenogenesis virgin females will give rise only to sons while impregnated ones will produce offspring of both sexes, the sex of the individual depending on whether it arises from a fertilized or from an unfertilized egg. In certain scale insects such as *Pseudaulacaspis pentagona* a different type of haplodiploidy exists – the male is haploid but arises from a fertilized egg, the paternal chromosomes being eliminated from the nuclei during certain of the cleavage divisions. Maleness in this case seems to result from a certain quality of the egg cytoplasm which causes elimination of the paternal chromosomes.

In another group of scale insects, the Iceryini, a more orthodox type of haplodiploidy exists. In members of this tribe there are two chromosomes in the males and four in the females. A single mitotic division replaces meiosis in the spermatogenesis. In several species, including the widespread *Icerya purchasi*, the females have been converted into hermaphrodites with an ovotestis whose ovarian portion is diploid, the testicular part being haploid as a result of a somatic reduction process

which takes place early in development. Males arise from unfertilized eggs in the Iceryini; the hermaphrodites are capable of self-fertilization but may mate with males occasionally.

A few species of Hymenoptera seem to have abandoned haplodiploidy for a wholly thelytokous method of reproduction, and in many social species the workers or some of them, instead of being entirely sterile, lay eggs which develop thelytokously.

Various authors have suggested that perhaps the Hymenoptera should be regarded as diploid-tetraploid rather than haploid-diploid. We shall never know which system existed in the earliest members of the Hymenopteran evolutionary lineage, but there can be no doubt that the males of modern Hymenoptera are both genetically and cytologically haploid. It has been claimed that *Diprion simile* is diplotetraploid, since it has twice as many chromosomes as other members of the genus, but this is doubtful (more probably it has doubled its chromosome number by 'dissociations'). The chromosome numbers of the Iceryine coccids and the haplodiploid mites are so low that it is most improbable that they arose as diplotetraploid forms.

One way of looking at haplodiploid forms such as the *Hymenoptera* is to regard them as having a multiple $X_1X_2 \ldots O$ mechanism of sex determination with no autosomes. Their evolutionary origin might be thought of as a progressive increase in the number of X's and a decrease in the number of autosomes. A species with $X_1X_2X_3X_4O$ in the male and a single pair of autosomes (no such form is actually known) might be well on the way to becoming a haplodiploid.

In sawflies, ants, wasps, etc., with only one nuclear division intervening between the primary spermatocyte and the sperm, each primary spermatocyte will obviously produce two spermatozoa. But in all true bees

(family Apidae) even this single division is an unequal one as far as the cytoplasm is concerned and gives rise to one functional spermatid and a small residual cell which does not form a sperm.

Automictic (meiotic) thelytoky

In thelytokous species of animals males are absent or non-functional in a genetic sense and females give rise to female progeny without fertilization taking place. In the automictic type of thelytoky a normal meiosis involving synapsis and chiasma formation takes place during the maturation of the egg and following numerical reduction the somatic number is restored by some kind of compensatory doubling of the chromosome set. In apomictic thelytoky, on the other hand, meiosis is totally absent. In neither case does any genetic recombination take place and in both types of thelytoky the offspring must be expected to be genetically almost completely identical to one another and to their mother. In some automictic systems the individuals must be almost completely homozygous while in apomictic thelytoky genetic heterozygosity must be expected to increase without limit in the course of generations.

Because of the absence of genetic recombination, the adaptive and evolutionary potentialities of both types of thelytoky must be strictly limited. Adaptive gene complexes can only arise by successive mutations in individual ancestral lineages and never by the combination in an individual of alleles present in two different parents. Thelytoky may be a strikingly successful genetic system in certain circumstances, because of the high reproductive potential which results from it, but it is likely to prove an evolutionary blind alley in the long run, because of lack of genetic plasticity and adaptability.

There are several sub-types of automictic thelytoky

according to the exact manner in which the somatic number is restored. The most widespread mechanism seems to be an ineffective second meiotic division. The division may be entirely suppressed, or the two daughter nuclei may come together again and fuse, to produce a compensatory doubling of the chromosome number. In another type of automictic parthenogenesis found in some moths the egg begins to develop with the reduced chromosome number, but after a few cleavage divisions the cleavage nuclei fuse in pairs. Finally, in some parthenogenetic earthworms the compensatory doubling of the chromosome number occurs just *before* meiosis instead of after it.

Species with automictic thelytoky are usually diploid, but some polyploid forms occur. Thus, for example, one race of the moth *Solenobia triquetrella* (in which the cleavage nuclei fuse in pairs) is tetraploid. The chromosomes, however, form bivalents, not quadrivalents, at meiosis. And many of the thelytokous species of earthworms are polyploid. But a triploid earthworm with a somatic chromosome number of 54, which doubles its chromosome number at the last oogonial division, will show 54 bivalents at meiosis, so that its triploid nature is not apparent from an inspection of the first meiotic division. Thelytoky of this general type is widespread in the earthworm genus *Allolobophora* but has not been reported in *Lumbricus*.

Apomictic (ameiotic) thelytoky

In this type of thelytoky meiosis is entirely suppressed. Usually no pairing of the chromosomes occurs at all and the egg goes through a single maturation division, which is a simple mitosis; but in the apomictic biotype of the roach *Pycnoscelus surinamensis* there are two maturation divisions, both mitotic in character. In this form it is stated that synapsis occurs, but the halves of

the bivalents fall apart after pachytene, when no chiasmata are formed.

Many thelytokous species have very wide geographic distributions and may extend far beyond the range of related bisexual forms. The parthenogenetic mantid *Brunneria borealis*, which occurs in the southern states of the U.S., is a member of a neotropical genus which includes several bisexual Brazilian species, and the apomictic Tettigoniid *Saga pedo*, which ranges from the Balkans to Spain, belongs to an eastern Mediterranean genus containing numerous bisexual species in Turkey, Israel and neighbouring countries. In both instances the parthenogenetic species have no doubt been able to extend their range beyond that of the rest of the genus because any single individual accidentally transported to a new locality can give rise to a population. But the rarity of these species, which seem to be confined to a very narrow range of habitats and have a very 'patchy' geographic distribution, is evidence of lack of genetic adaptability.

There are a number of species of animals in which reproduction is truly thelytokous, but sperms are essential for 'triggering' the development of the egg, even though they do not fertilize it in a genetical sense. The thelytokous individual may be a hermaphrodite, producing sperms as well as eggs; or the sperms may be derived from males of the same species or a different one. Particularly striking are those cases where the sperm of a different species or related bisexual strain is employed. The spider beetle *Ptinus latro* consists exclusively of triploid females that reproduce parthenogenetically. But they only produce progeny after mating with the diploid males of a related bisexual species. A similar situation, except that triploidy is not known to be involved, exists in the little Mexican-Texan fish *Mollienisia formosa* which reproduces entirely by

parthenogenesis but the eggs will only develop after they have been fertilized by sperm from one or other of two related species, *M. sphenops* and *M. latipinna*. Many generations of 'back-crossing' to these species does not lead to any replacement of the characters of *M. formosa*, so that we may be sure that the sperm does not transmit chromosomes to the offspring. This type of genetic system is known as *pseudogamy*; it is also known in a number of nematode and earthworm species and in the moth *Luffia lapidella*. A similar system occurs in some species of plants. The sperms which function in pseudogamous reproduction do not require a complete set of chromosomes, so that the spermatogenesis of earthworms or flatworms which reproduce by this method may lack a regular meiosis and show a more or less chaotic distribution of the chromosomes to the developing sperms. Very complicated chromosome cycles have been described in the various races of the pseudogamous planarian *Dugesia benazzii* from the island of Sardinia and some related forms.

Parthenogenesis has been studied in a number of Dipteran species with polytene chromosomes. It occurs, occasionally, in *Drosophila parthenogenetica* and, regularly, in a certain strain of *D. mangabeiri*. The fly *Lonchoptera dubia* apparently consists of four different biotypes, each heterozygous for a different combination of chromosomal inversions. In *D. mangabeiri*, also, parthenogenesis operates in such a way as to ensure permanent inversion-heterozygosity, no structural homozygotes being produced. In these cases the cytogenetic mechanism presumably makes use of heterosis based on heterozygous inversion sequences. It is characteristic of *Lonchoptera* and some parthenogenetic Simuliids which have been studied cytologically that the same heterozygous combinations of inversion sequences have a very wide geographic distribution.

Most species that reproduce regularly or exclusively by automictic parthenogenesis are diploid. Typical examples are the white fly *Trialeurodes vaporariorum*, the Ichneumonid *Nemeritis canescens* and the sawfly *Pristiphora pallipes*. A few animal species with automictic parthenogenesis are polyploid (certain races of *Artemia salina* and of the moths *Solenobia triquetrella* and *S. lichenella*), but they are even-numbered polyploids (4*n*, 6*n*, 8*n* . . .) and no multivalents seem to be formed at meiosis. Only in the parthenogenetic earthworms, with their peculiar pre-meiotic doubling of the chromosome number, is triploidy, pentaploidy, etc., compatible with normal meiosis in an automictic system. It is not clear why these earthworm species never form multivalents; possibly the chromosomes which undergo synapsis are always 'sisters' from the last pre-meiotic mitosis.

Automictic parthenogenesis seems to be very rare in plants, where a bewildering variety of types of apomixis are known. Apomictic plants are frequently polyploids of hybrid origin. They may form *agamic complexes* composed of very numerous biotypes, taxonomically difficult (examples occur in the genera *Crepis*, *Hieracium*, *Poa*, *Potentilla*, *Rubus*). In species with apomictic parthenogenesis all 'meiotic' barriers to polyploidy, including odd-numbered polyploidy (3*n*, 5*n* . . .) and aneuploidy, are of course absent. In other words, since the karyotype does not undergo meiosis it can be of a type which could not undergo a normal meiosis. As examples of polyploid apomicts among animals we may mention the French isopod *Trichoniscus coelebs* which is a triploid and the Mediterranean Tettigoniid *Saga pedo* which is a tetraploid. Among 17 species or races of apomictic weevils belonging to the subfamilies Otiorrhynchinae and Brachyderinae one is a diploid, eleven are triploids, four are tetraploids and one is a

pentaploid. It seems probable that the odd-numbered polyploids have arisen by occasional crossing between normally parthenogenetic strains and related bisexual diploids; the situation may be basically similar to that found in certain agamic complexes in plants, but considerably simpler.

In many cases what appears to be a single species morphologically includes both parthenogenetic and bisexual biotypes, between which gene exchange is impossible (it may have occurred, however, at some time in the past when the thelytokous reproduction of the parthenogenetic biotype was less firmly established). Such parthenogenetic and bisexual biotypes may occur together in the same geographic regions but perhaps more frequently occupy different areas. Thus the Chrysomelid beetle *Adoxus obscurus* exists as a sexually reproducing diploid in North America and as a parthenogenetic triploid in Europe. The brine shrimp *Artemia salina* and the greenhouse white fly *Trialeurodes vaporariorum* are classic instances of 'superspecies' which include both bisexual and parthenogenetic strains.

Cyclical parthenogenesis

In view of the obvious *reproductive* advantages of thelytoky (high 'intrinsic rate of increase') and the *genetic* advantages of sexual reproduction, it is hardly surprising that whole groups of organisms have developed genetic systems in which the two are combined in cyclical alternation of one kind or another. We may consider three types of such alternation. (1) In the midge *Miastor metraloas* the larvae reproduce thelytokously under the bark of rotting logs, feeding on fungal mycelium. This can go on for an indefinite number of generations, but when conditions become unfavourable, male and female flying midges are produced, which

reproduce sexually. (2) In many gall wasps (Cynipids) there are always two generations a year, one sexual, the other thelytokous. The males of the sexual generation are haploid, as in other Hymenoptera. (3) In most aphids an indefinite number of thelytokous generations during the warmer part of the year are followed by a single sexual generation in the fall or winter.

Animals with cyclical parthenogenesis must obviously have chromosomal systems that are capable of going through a normal meiosis, occasionally. They are consequently all diploids, as far as known. A variety of genetic mechanisms are responsible for the production of male and female 'sexual' individuals from a population that has previously been reproducing thelytokously.

Aphids, cynipids and *Miastor* all lack Y-chromosomes, and it does not seem possible that a mechanism of cyclical parthenogenesis could exist in which the males were XY.

In addition to the aphids, gall wasps and a few species of midges, the Cladocera, rotifers and digenetic trematodes all exhibit cyclical parthenogenesis in most of their species (in the latter the sexual individuals are the hermaphroditic 'adult' flukes). But the cytology of rotifers and trematodes is not well known.

Many species that do not usually or normally reproduce parthenogenetically have some ability to do so if females are kept unmated. In grouse-locusts (Tetrigidae) and other grasshoppers, eggs of virgin females may develop into females through failure of the second meiotic division; the homologous chromosomes may lie side by side in the embryonic divisions, unlike the situation in bisexually produced embryos. But in other instances development of haploid eggs may occur, with more or less incomplete 'diploidization' through fusion of cleavage nuclei in pairs.

CHAPTER X

Chromosomal Polymorphism in Natural Populations

The natural populations of many species of organisms are polymorphic for various kinds of chromosomal rearrangements. It is difficult to form an adequate idea of the extent of such polymorphisms. They occur in about 80% of the species of *Drosophila*, perhaps in a similar fraction of *Chironomus* species, and in at least 10–20% of grasshopper species. Sporadic instances in other groups merely provide a minimum estimate of the frequency of chromosomal polymorphism, many kinds being difficult to detect cytologically.

The main types of chromosomal polymorphism which occur in natural populations are as follows:

1. Inversions (*a*) paracentric
   (*b*) pericentric
2. Translocations (*a*) mutual ('interchanges')
   (*b*) centric fusions
   (*c*) dissociations
3. Differences in amount of genetic material in a chromosome (*a*) duplications
   (*b*) deficiencies
4. Supernumerary chromosomes.

Chromosomal rearrangements cannot as a rule be expected to establish themselves in natural populations if they reduce the fertility of individuals heterozygous

for them to any significant extent. Meiosis presents various complexities in individuals heterozygous for some kinds of chromosomal rearrangements. In the case of heterozygotes for inversions it seems to be usual for the mutually inverted segments to twist around so as to form a 'reversed loop', with all loci except perhaps

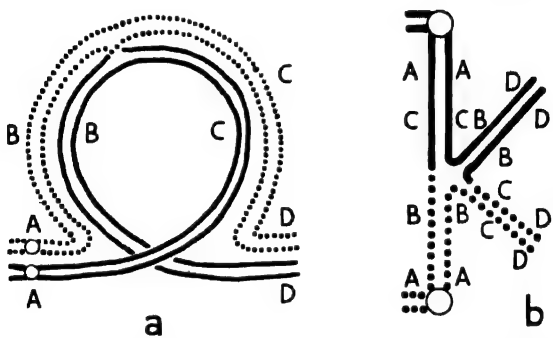


Fig. 21. Diagrams of a meiotic bivalent heterozygous for a pericentric inversion

Paternal strands black, maternal ones dotted. A single chiasma is shown within the inversion loop. *a*, diplotene; *b*, first metaphase showing the dicentric ACBA strand and the acentric DBCD one.

those immediately adjacent to the ends of the inversion accurately synapsed (Fig. 21). Chiasmata may now arise within the reversed loop as well as outside it. The consequences, in the case of paracentric and pericentric inversions, are different. In an individual heterozygous for a paracentric inversion, a single chiasma in the loop will give rise to a dicentric and an acentric strand. In the oogenesis of *Drosophila* and the midge *Sciara* (and probably in many other organisms) the

dicentric and acentric strands are left in the polar nuclei (which are destined to degenerate in any case) and the egg nucleus receives a monocentric strand. Since in these genera no chiasmata are formed in spermatogenesis, they do not suffer any significant loss of fertility on account of heterozygosity for paracentric inversions. A 'double defence mechanism' protects them from the cytogenetic consequences that would otherwise result from this type of structural heterozygosity. Polymorphism for paracentric inversions is, in fact, very common in many species of *Drosophila* and some of *Sciara*, as many as 45 different inversions being known in such species as the neotropical *D. willistoni* and the European *D. subobscura*. We leave it to the reader to work out the consequences of two or more chiasmata in an inversion loop or between it and the centromere, which vary according to which of the four strands are involved. As far as *Drosophila* is concerned, however, this is largely a theoretical exercise, since the chiasma frequency is not high enough or the naturally occurring inversions long enough, for such multiple chiasmata in the inversion or proximal to it, to nappen at all frequently.

In the midges of the genus *Chironomus* and in a few other genera of Diptera paracentric inversions seem to be quite common in the natural populations of certain species, in spite of the fact that chiasmata *are* formed in spermatogenesis. Thus we might expect such species to form a significant number of sperms with broken or acentric chromatids which would kill the eggs they fertilized. Apparently this does not happen; the chromatid bridges do not break at first anaphase and the secondary spermatocytes held together by such bridges merely form giant spermatids which are genetically harmless, since they are incapable of taking part in fertilization. The slight reduction in the number of

functional sperms formed is probably quite unimportant. Thus *Chironomus*, like *Drosophila* and *Sciara*, avoids paying the penalty for paracentric inversion heterozygosity, although the mechanism, as far as the male is concerned, is quite different (the mechanism in the female is probably the same).

The consequences of a chiasma in the inversion loop will be quite different in the case of heterozygosity for a pericentric inversion. No acentric or dicentric chromatids will be produced, but the two crossover strands will each be deficient for one terminal segment and have the other in duplicate. *Drosophila* seems to have no mechanism to 'protect' it from the loss of eggs carrying such duplication-deficiency chromosomes. It is hence not surprising that virtually all the inversions met with in natural populations of *Drosophila* species are paracentric, pericentric ones being almost unknown (the two types presumably arise with equal frequency, but the pericentric ones are eliminated by natural selection).

In certain species of grasshoppers, however, and in a few other insects heterozygosity for pericentric inversions is quite common. Apparently in these species 'reversed loops' are simply not formed at zygotene, so that no chiasmata can be formed between the mutually inverted segments and hence no chromatids with deficiencies and duplications are produced.

Apart from Diptera and grasshoppers, we have almost no information on the extent to which other groups of animals are 'protected' from the consequences of inversion-heterozygosity. Various plants such as *Trillium kamtschaticum*, *Paris quadrifolia* and *Paeonia* species have been recorded as showing dicentric and acentric chromatids at meiosis which were the result of crossing-over between mutually inverted segments. This probably leads to a reduction in fertility, but the species in question are mostly ones in which some form

of clonal or vegetative reproduction exists, so that a loss of sexual fertility may not be very important to them.

Heterozygosity for a mutual translocation ('interchange') will lead to a serious loss of fertility unless a ring or chain of four chromosomes is regularly formed at meiosis and orientates itself on the meiotic spindle so that the alternate chromosomes invariably pass to opposite poles at first anaphase. In animals interchange-polymorphism exists in the roach *Periplaneta americana* and in a number of species of South American scorpions belonging to the genera *Tityus* and *Isometrus*, where several interchanges may co-exist in the same population. The chromosomes of these scorpions seem to possess multiple or diffuse centromeres. Their genetic systems are highly peculiar, since in some instances rings containing uneven numbers of chromosomes are formed at meiosis.

The genetic significance of inversion-polymorphism in natural populations depends on the fact that crossing-over between the mutually inverted segments in structurally heterozygous individuals is effectively suppressed, either because it simply does not occur (pericentric inversions of grasshoppers) or if it does take place (paracentric inversions in *Drosophila* females and *Chironomus* males) the crossover chromatids do not pass into the functional gametes. On the other hand, crossing-over will lead to free genetic recombination in individuals homozygous for inversions.

As a result of this suppression of crossing-over in the heterozygotes, the mutually inverted regions are genetically isolated from one another, and must be expected to accumulate different combinations of alleles in the course of evolution.

It has been shown in a number of instances that this process of genetic evolution involves a coadaptation

between the allelic contents of the relatively inverted sections which results in the heterozygotes having either a higher viability or fecundity than either of the two classes of structural heterozygotes. It is this selective superiority of inversion heterozygotes (*heterosis*) which maintains an equilibrium in the population, the relative frequency of the two alternative sequences being determined by the ratio of the selective values of the two kinds of homozygotes. In a few extreme cases the inversion homozygotes are so ill adapted that we have an approach to a *balanced lethal* condition; but this is probably quite unusual in natural populations. In general, heterosis is still manifested in artificial laboratory colonies maintained in population cages. But the genetic properties of a particular cytological inversion sequence will not be the same at two localities situated some considerable distance apart, so that artificially produced inversion heterozygotes which have one chromosome derived from locality 'A' and the other member of the pair from locality 'B' will not necessarily exhibit heterosis. In such a case an equilibrium will not necessarily be established in the cage and one sequence may entirely replace the other in the course of a number of generations of natural selection.

The adaptive significance of other types of chromosomal polymorphism such as translocations, deletions and duplications, where these exist in a state of flux in natural populations, is probably similar to that of inversions, i.e. the genetic equilibrium which is established depends on the heterozygotes having a higher selective value than either homozygous type. The higher selective value of the heterozygotes may depend on superior viability or fecundity or both. However, heterosis is not the only principle on which a stable equilibrium can depend. If there is an inverse relationship between the selective values of the homozygous

genotypes and their frequency in the population, so that a particular type of chromosome is favoured by natural selection when rare and discriminated against when common, an equilibrium will be maintained. It is still rather uncertain how important this principle is in natural populations.

In the genus *Drosophila* there are some species which exhibit a great deal of inversion polymorphism, so that the average individual in a wild population is heterozygous for several or many inverted chromosome sections. Typical of such species are the European *D. subobscura* and the neotropical *D. willistoni*, in each of which about 45 different inversions (by comparison with an arbitrary 'standard' sequence) are known. Other *Drosophila* species show only a few inversions or none at all. In general most of the species with many inversions are common, ecologically dominant forms, but the ones lacking inversions include both rare, ecologically specialized species (*D. novamexicana*) and common widespread ones (*D. repleta*). In some species with many inversions these seem to be distributed pretty much at random over the chromosome set (*D. willistoni*) while in others (*D. pseudoobscura*, *D. melanica*) there is a strongly marked tendency for most or all the inversions to be in one chromosome pair.

In grasshoppers, likewise, some species such as *Trimerotropis thalassica*, *T. suffusa* and *T. sparsa* (all species of the western U.S.) show many pericentric inversions, while others (*T. maritima*, *T. citrina*) lack inversions altogether. Some species such as *T. pallidipennis*, which do not exhibit inversion polymorphism at the present time, possess karyotypes which, by comparison with those of related species, prove that they were polymorphic for inversions at some time in the past. It is not usual in grasshoppers for more than one pericentric inversion to establish itself in the case of

each chromosomal element. Thus individual elements may be dimorphic or occasionally trimorphic, but only in the case of the 'CD' chromosome of the Australian *Moraba scurra* are four different sequences known to exist, and no more than three of these co-exist in any one population. The vast majority of grasshopper species are definitely *not* polymorphic for pericentric inversions. Supernumerary chromosomes and chromosome regions (see p. 146) are much commoner forms of cytological polymorphism in this group of insects.

In several instances in *Drosophila* it has been shown that there is a peculiar type of *organization effect* in a chromosome, i.e. two inverted sections have to be present in the *cis* configuration (i.e. in the same homologue) to produce a highly viable individual; if the same inversions are present in the *trans* or *repulsion* configuration (one in one homologue, one in the other) an ill-adapted or relatively inviable fly results. And in the grasshopper *Moraba scurra* a complex genetic interaction exists between two inversion systems located on different chromosome pairs.

Obvious phenotypic effects of inversions have not been detected, although they may exist in some species with conspicuous colour or pattern polymorphisms as well as inversion polymorphism. In *Moraba scurra* certain cytological gene sequences are known to produce large phenotypes, while others have size-decreasing effects.

Polymorphism for mutual translocations has been studied especially in certain groups of plants. In addition, many translocations produced by radiation have been studied in maize and other species.

Certain species of *Paeonia* and *Clarkia* have their natural populations regularly polymorphic for translocations. In such plants rings and chains of four six and eight chromosomes are encountered at meiosis,

the orientation of the chromosomes being regularly zig-zag at first metaphase. The widespread occurrence of translocation polymorphism in these species suggests that the heterozygotes have a selective advantage. This type of genetic system has been carried a stage further in certain plants (*Rhoeo discolor*, *Hypericum punctatum*, many species of *Oenothera*) in which heterozygosity for translocations has become obligatory, the structural homozygotes being either not formed at all or inviable (see p. 167).

With the exception of the scorpion species previously mentioned, with their polycentric chromosomes, the only translocations which seem to stand some chance of establishing themselves in natural populations (apart from centric fusions and dissociations, which are accompanied by deletion or duplication of short segments) are those which occur between metacentric chromosomes with approximately equal arms and with distal localization of chiasmata. Translocations between other types of chromosomes, or between chromosomes of quite different sizes, are unlikely to give the zig-zag orientation at first metaphase with sufficient regularity to ensure high fertility. Translocations of minute distal regions with very low chiasma frequencies may be quite common but are necessarily undetectable in most cases. We shall deal with the occurrence of centric fusions and dissociations in natural populations in the next chapter, in connexion with the general problem of chromosomal rearrangements in speciation and evolution.

Wild populations of many animal and plant species contain, in addition to the chromosomes of the regular set, certain *supernumerary chromosomes* (sometimes called B-chromosomes). By definition, these are chromosomes which are lacking in at least some (and usually most) of the individuals. Thus we have a population

consisting of individuals with 0, 1, 2, 3 . . . supernumerary chromosomes.

Most supernumerary chromosomes are largely or entirely heterochromatic, but some, such as the B-chromosomes of maize, contain sizeable euchromatic segments while others have been described as being entirely euchromatic.

Supernumerary chromosomes do not have conspicuous effects on the visible phenotype of the individual, but we may be confident that they do influence viability, growth rates and fecundity in various subtle ways. Their survival in natural populations is most readily explicable if they have a beneficial effect in small doses (i.e. when only one or two are present) and a deleterious one when they are too numerous.

Supernumerary chromosomes are unstable in various ways. Species possessing them frequently exhibit several different kinds, due to structural changes such as deletions and duplications having occurred. Many supernumeraries are isochromosomes. Some tend to be lost from the somatic cells, although preserved in the germ track. In rye and *Anthoxanthum* both daughter supernumeraries regularly get included in the generative nucleus at the first microspore division, and in rye there is a similar behaviour in the comparable division of the embryo-sac. In maize the B-chromosomes behave in an identical manner at the second microspore division. In all these cases their inheritance deviates from Mendelian rules.

Some supernumeraries exhibit regular bivalent formation at meiosis, when there are two of them, but others are usually univalents, regardless of how many are present.

The origin of supernumerary chromosomes is not known in detail in any single instance; but it is clear on general grounds that they must arise from members of

the regular complement. The first stage is probably the formation, by deletion, of a small fragment chromosome composed in most cases largely or entirely of heterochromatin. Repeated evolutionary duplication of segments of such an element may convert it into a large supernumerary chromosome.

There is no evidence that supernumerary chromosomes may eventually become indispensable to the individual and hence evolve into members of the regular chromosome set. But supernumerary elements may on many occasions have acted as donors of centromeres and telomeres in the process of evolutionary increase in chromosome number by dissociation (see p. 94).

The frequency of supernumerary chromosomes in natural populations ranges from approximately 1 per 500 individuals to 3 or 4 per individual (in the latter case individuals lacking supernumeraries may be quite scarce).

Apart from supernumerary chromosomes, the natural populations of many species contain *heterochromatic chromosome regions* existing as *supernumerary segments* of one or more members of the regular chromosome set. There does not seem to be much difference in principle between such segments and typical supernumerary chromosomes except that a supernumerary segment of an essential chromosome is necessarily inherited in an orthodox Mendelian manner and that each such segment may be present 0, 1 or 2 times in a diploid organism but not 3, 4 . . . times. Some supernumerary heterochromatic segments form virtually entire chromosome arms, while others may be inserted into or attached to chromosome limbs that are otherwise euchromatic. Individuals that are heterozygous for supernumerary regions naturally show *unequal bivalents*, made up of a large and a small chromosome, at meiosis.

Supernumerary chromosomes and chromosome regions may be rather readily interconvertible in evolution, since there is a tendency for them to co-exist in the same species.

Chromosomal polymorphism may be looked upon as one way in which the genetic variability of a species may be canalized. In several instances it has been shown that inversion polymorphism reaches a maximum in the centre of the territory occupied by a species, peripheral populations being less polymorphic, or even cytologically monomorphic. Cytological polymorphism seems to be rare in vertebrates, almost the only well-authenticated instance being that of the shrew *Sorex araneus* (see p. 157). The possibility exists, however, that many species lacking polytene chromosomes may be polymorphic for paracentric inversions which would be virtually undetectable by the usual cytological techniques if they did not give rise to 'reversed loops' at zygotene in the heterozygotes, but merely acted as 'total crossover suppressors'. However, such a hypothetical possibility seems more likely in grasshoppers, where pericentric inversions are known to behave in this manner, than in mammals, where heterozygosity for pericentric inversions is unknown in natural populations.

CHAPTER XI

Chromosomal Changes in Evolution

Although the general theory of the mechanism of evolution is outside the scope of this book we cannot leave the subject of the chromosomes without considering the changes which chromosome sets have undergone in the course of evolution and the relation of these changes to speciation.

In taxonomic groups where only the gross morphology of the chromosomes can be studied, it frequently happens that two or more closely related species appear to have identical karyotypes. It is uncertain in such cases whether cytological differences might be revealed if a more detailed analysis (such as is provided by the polytene chromosomes of the Diptera) were possible. In the genus *Drosophila* the polytene chromosomes of *D. mulleri*, *D. aldrichi* and *D. wheeleri* do not seem to show any visible differences; but this seems to be a rather exceptional case in *Drosophila*, since a considerable number of instances are known where species which can hardly be distinguished externally show very profound cytological differences in their polytene chromosome sets. Thus the evidence from *Drosophila*, which is supported by less complete evidence from various other genera of Diptera in which detailed studies of the salivary gland chromosomes have been carried out (Chironomidae, *Sciara*, *Simulium*, *Anopheles*), suggests that the process of speciation usually

involves or includes some chromosomal changes, although this is probably not invariably so.

Cytological differences between species can be detected by simply comparing drawings or photographs of the karyotypes of the two forms; but it is greatly facilitated if F_1 hybrids can be studied. Thus the most significant studies in cytotaxonomy have been based on analyses of the meiosis or polytene chromosomes of interspecific hybrids.

Apart from cases of differences in chromosome number which are due to polyploidy, cytotaxonomic differences between the karyotypes of related species must have arisen by chromosomal rearrangements, such as inversions, translocations, deletions and duplications, or combinations of these. Where two species are each homozygous for a different arrangement, so that their F_1 hybrid is structurally heterozygous, it seems fairly clear that there was an evolutionary stage when the rearrangement existed as a polymorphism in an ancestral population. Thus we might generalize and say that the cytotaxonomic differences of the present time represent the remains, as it were, of chromosomal polymorphisms that existed in the past, one sequence having reached fixation in one evolutionary lineage, the other sequence in a second line of evolution.

In the higher plants, where a considerable number of the species are polyploid, cytotaxonomic studies have been largely concerned with understanding the origin of species that have arisen by allopolyploidy, i.e. through the addition of chromosome sets derived from two or more original species. In animals, on the other hand, polyploidy seems to have played almost no part in the evolution of bisexual species, and only a minor role in those groups, such as the Turbellaria, where hermaphroditism is the rule; it is only when we come to forms which reproduce parthenogenetically (see

Chapter IX) that we encounter many polyploid biotypes.

In most groups it can be shown that there is a close parallelism between the kinds of chromosomal rearrangement encountered as intra-population polymorphisms and as cytotaxonomic differences between species. Thus in the genus *Drosophila* considered as a whole paracentric inversions are by far the commonest type of cytological polymorphism within species and are also the main kind of chromosomal difference between species. In certain groups of closely related species of *Drosophila* (e.g. the *repleta* group) relatively few such inversions seem to have established themselves, so that cytological polymorphism only attains a low level and cytological differences between species are minimal; while in other sections of the genus (e.g. the forms related to *D. montana* in the *virilis* group) inversion polymorphism reaches a high level of complexity and the various races and species are likewise distinguished by many inversion-differences.

An apparent exception to the above general principle exists, however, in the case of the mammals (especially the rodents, which have been extensively studied) where extreme differences frequently exist between the karyotypes of related species but where few or no instances of cytological polymorphism in natural populations are known. Future work may show whether this discrepancy is a real one; it is quite possible that more instances of chromosomal polymorphism would be found in mammals if they were searched for. However, many cytotaxonomic differences between mammalian species may have arisen by rearrangements which established themselves in small isolated populations without the heterozygotes being necessarily heterotic.

An important distinction may be drawn between those chromosomal rearrangements such as inversions

and mutual translocations which leave the total amount of genetic material the same and the duplications and deletions which lead to increases or decreases. In *Drosophila* most deletions are lethal in the homozygous state unless they are very short (1–2 bands in the polytene chromosomes). Deletions of considerably longer sections of heterochromatin may, however, be compatible with viability. Duplications likewise affect the genic balance of the organism and are seldom likely to be tolerated in natural populations unless they are very short or are in heterochromatic regions of the chromosomes.

There is evidence, however, that duplications of chromosomal regions have occurred in the phylogeny of the genus *Drosophila*, possibly at long intervals in the remote past. In the polytene chromosomes it is possible to recognize a certain number of 'reversed repeats', i.e. regions where a particular sequence of bands occurs more than once but in a reversed order (as in the sequence of letters *abcdeffedgh*). And it is possible that the so-called 'doublets' represent evolutionary duplications of single bands that have occurred long ago in evolution.

Direct evidence of deletions in evolution is harder to obtain, but over a long period of phylogeny it is clear that an approximate equilibrium between evolutionary duplications and deletions must exist. The usual sequence of events is probably for euchromatic regions to become gradually converted into heterochromatin and then eventually deleted.

Where two related species of bisexual animals differ in chromosome number (i.e. excluding hermaphroditic and parthenogenetic species where the possibility of polyploidy has to be considered) it is usual to invoke chromosomal 'fusion' or 'fragmentation' to account for the difference in number. The laws and principles

governing such events are, however, rather more precise than many authors have considered in the past. The most important restrictions are (1) that in most groups each chromosome must contain a single centromere (we have already noted that in some exceptional groups chromosomes with multiple centromeres exist) and (2) that in all cases a chromosome must have a telomere at each end in order to be self-perpetuating at mitosis.

Little or no evidence exists that evolutionary changes in chromosome number have come about in animals through duplication or deletion of whole chromosomes. The situation may be somewhat different in groups where high grades of polyploidy occur and where duplication or deletion of a single chromosome does not upset the genetic balance of the organism to the same extent as in a diploid.

When the karyotypes of related species of animals are compared it is frequently found that a pair of metacentric chromosomes in one species seems to be represented by two pairs of acrocentrics in another. Such relationships were first studied by Robertson in grasshoppers – they have subsequently been noted in almost all taxonomic groups where both acrocentric and metacentric chromosomes exist. In higher plants they are much less common, acrocentric chromosomes being very rare except in a few families.

A relationship of this kind implies one of two alternatives. Either a chromosomal 'fusion' of two acrocentrics to produce a metacentric element has occurred in evolution; or a 'dissociation' of a metacentric to give two acrocentrics has happened. In the true grasshoppers (family Acrididae), the overwhelming number of species have 12 pairs of acrocentrics and only single species or genera show 10 acrocentrics plus 1 metacentric, 8 acrocentrics plus 2 metacentrics or 6 acrocentrics plus 3

metacentrics. In this case we can be reasonably certain that 'fusions' have occurred. Such fusions (or *centric fusions*, to emphasize the fact that it is the proximal ends which become joined) probably come about by various kinds of extremely unequal translocations, a small piece of each of the original acrocentrics being lost in the process (Fig. 16). Dissociations have also occurred in the karyotype evolution of a number of groups. Obviously, if a metacentric were simply to break somewhere near the middle, one fragment would be left without a centromere and both would have freshly broken ends which would not be expected to survive unchanged. It is therefore probable that dissociations are likewise a special kind of translocation in which some other chromosome donates a centromere and two telomeres (Fig. 16). In one critical case it has been proved that this is, in fact, what has occurred.

Centric fusion thus involves the loss of some chromosomal material adjacent to the centromeres while dissociation leads to the reduplication of short regions. Since the regions on either side of the centromere are commonly heterochromatic they are perhaps relatively unimportant, genetically, so that loss or duplication of short segments does not lead to impaired viability.

Centric fusions and dissociations can exist in a polymorphic state in natural populations, so that we have a *chromosome number polymorphism*. Striking instances have been described in the mollusc *Thais lapillus*, the beetle *Chilocorus stigma* and the shrew *Sorex araneus*. Alternatively we may have two races of a species, one homozygous for the fusion (or dissociation), the other for its absence; the races may overlap in geographical distribution widely or narrowly. The Australian grasshopper *Moraba scurra* has two races, one with $2n$ ♂ = 15, the other homozygous for a dissociation so that $2n$ ♂ = 17; the zone of overlap, before destruction of

the natural habitat by grazing, was probably at most a few hundred yards wide. Hybrids between the two races show a characteristic trivalent at meiosis, consist-

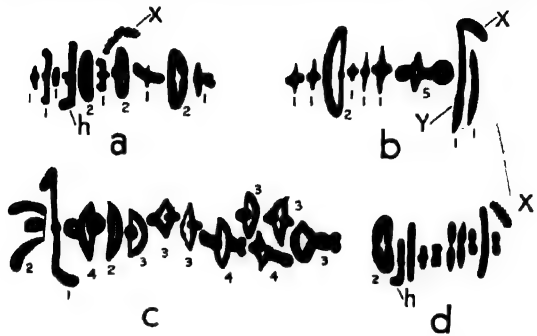


Fig. 22. First meiotic divisions of various species of animals in side-view

a, the North American grasshopper *Trimerotropis gracilis* (XO, $2n\ ♂ = 21$), showing one bivalent (*h*) heterozygous for a pericentric inversion. *b*, an Australian grasshopper *Tolgadia* sp. (XY, $2n\ ♂ = 18$); this species has a karyotype derived from the primitive $2n\ ♂ = 23$ one by two fusions between autosomes and one fusion between the X and an autosome. *c*, the European salamander *Triturus cristatus carnifex* subsp. ($2n = 24$). *d*, the Australian grasshopper *Austroicetes interioris* ($2n\ ♂ = 21$); this individual is heterozygous for a pericentric inversion in one bivalent. In *c* all the chromosomes are metacentric; *a* has a metacentric X, 11 metacentric autosomes and 9 acrocentric ones; *b* has a metacentric neo-X, 4 metacentric autosomes and an acrocentric neo-Y. In the case of some of the bivalents the number of chiasmata is indicated by figures alongside them.

ing of two acrocentric chromosomes paired with a metacentric.

Similar cases are known in a number of other grasshopper species. The narrowness of the zone of overlap

suggests that the chromosome number heterozygotes are not adaptively superior and in fact are probably adaptively inferior to both homozygous genotypes. In instances of this kind we are apparently dealing with chromosomal rearrangements that were able to establish themselves in the first place because they gave rise to an adaptive superiority in the homozygote and in spite of the fact that they were actually disadvantageous when present in the heterozygote. The establishment of such a rearrangement would only be likely in a small peripherally isolated colony of a species whose powers of dispersal were poorly developed; otherwise, even if a colony of individuals homozygous for the rearrangement arose it would be liable to be swamped by immigrants, leading to the production of inferior heterozygotes.

In the beetle *Chilocorus* and the shrew *Sorex araneus*, referred to earlier, chromosome number polymorphism is geographically widespread, instead of being confined to a narrow zone of contact between two races. Thus in these cases the rearrangements have presumably been able to establish themselves as a result of heterozygote superiority rather than homozygote superiority. The case of *Thais lapillus* is in some respects intermediate and is peculiar in several ways. Cytologically homozygous races with $n = 13$ and $n = 18$ are ecologically rather than geographically separated in two littoral zones of the French coast. In an ecologically intermediate zone populations polymorphic for chromosome number exist.

In *Sorex araneus* there are three chromosome number polymorphisms, so that there are 3^3 or 27 cytologically distinguishable types of individuals in the population and the chromosome number ($2n$ ♂) ranges from 21 to 27.

In the genus *Drosophila* the main kinds of cytological

events that have occurred in evolution seem to have been paracentric inversions, gains or losses of heterochromatic segments, occasional centric fusions, and very rare pericentric inversions. There is no critical evidence of any dissociations having occurred and translocations other than centric fusions seem to have been virtually absent from the picture. It has been estimated that the number of paracentric inversions that have established themselves in the phylogeny of the genus *Drosophila* (over 600 species) may have been over 36,000.

In grasshoppers technical difficulties preclude estimates of the numbers of paracentric inversions that have established themselves; but it is probable that they have been very rare, by comparison with *Drosophila*. Pericentric inversions, on the other hand, have been relatively frequent in certain North American genera (*Trimerotropis*, *Circotettix*, *Aerochoreutes*) and in the Australian subfamily Morabinae. In both groups and in some other grasshopper genera, centric fusions have occurred in addition and in the Morabinae there is also evidence of dissociations. The cytologically aberrant South American grasshopper species *Dichroplus silveiraguidoi*, with $n = 4$, has apparently acquired 8 centric fusions and 6 pericentric inversions since it diverged from the main lineage of *Dichroplus* species with $n = 12$.

There are some groups of the animal kingdom in which chromosome numbers are very constant, while in others wide variation in chromosome number occurs. But even in groups where great constancy is the rule it is usually found that when a sufficiently large number of species are examined some of them have aberrant chromosome numbers. Almost all the Pentatomid bugs have $n = 7$, but one species is known with $n = 3$ and one with $n = 14$. Twenty species of Corixidae have $n = 12$, but

one species with $n = 13$ is known. However, all the species of mosquitoes thus far investigated (genera *Culex*, *Aedes*, *Anopheles* and *Theobaldia*) have three pairs of chromosomes. As examples of groups in which chromosome numbers are usually variable we may mention certain butterfly genera such as *Erebia* (species or races with 8, 10, 11, 12, 14, 15, 17, 19, 21, 22, 25, 28, 29, 40 and 51 pairs of chromosomes) and *Lysandra* (species or races with $n = 23$, 45, 82, 88, 90, 124–5, 131–50, 190–1, are known). There seems no need to invoke special mechanisms such as polyploidy or polysomy to account for such evolutionary changes in chromosome number. They have most likely arisen by the usual processes of fusion and dissociation which have been studied in other groups such as *Drosophila* and grasshoppers, where changes of chromosome number have been much fewer. *Lysandra nivescens*, the animal species with the highest known chromosome number, has probably undergone no less than 168 dissociations since it diverged from the main stock of the family with $n = 23$ or 24.

One instance that has given rise to much discussion is that of the hamsters (Rodentia-Cricetinae). The species *Cricetulus griseus* and *Cricetus cricetus* have $n = 11$ while *Mesocricetus auratus* has $n = 22$. It was accordingly claimed that the latter was a tetraploid and even an allotetraploid derived from two 11-chromosome species. All critical evidence is against this interpretation. A chromosome number of $n = 22$ is 'normal' for the subfamily, while one of $n = 11$ is highly unusual. It is hence the species with the lower numbers which are in need of explanation. A whole series of species in the genera *Cricetus* and *Cricetulus* have now been found with numbers between 11 and 22, thus connecting the two extremes. The amount of DNA in nuclei of *auratus* and *griseus* is not significantly different, a fact which is

difficult to reconcile with the polyploidy hypothesis. Uncritical acceptance of the idea that polyploidy has occurred in this or that group of bisexual animals by biologists who have made no detailed study of the evidence has unfortunately become far too common.

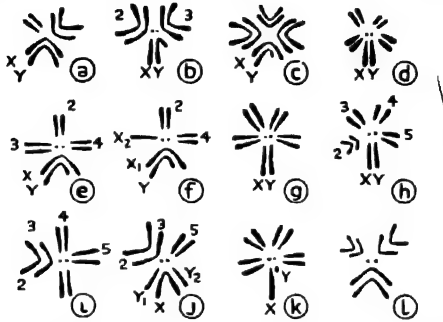


Fig. 23. Mitotic metaphase chromosomes of various species of Drosophila

All figures are of males, with the sex chromosome pair at the bottom of the figure. *a*, *D. willistoni*, *b*, *D. melanogaster*, *c*, *D. ananassae*, *d*, *D. subobscura*, *e*, *D. pseudoobscura* and *D. persimilis*, *f*, *D. miranda*, *g*, *D. virilis*, *h*, *D. montana*, *i*, *D. americana texana*, *j*, *D. americana americana*, *k*, *D. repleta*, *l*, *D. robusta* (redrawn from Patterson and Stone, 1952).

In many animal species centric fusions have occurred which involve the sex chromosomes. Some of these have converted species with an XO : XX sex chromosome mechanism into XY : XX forms, while others have converted XY : XX systems into more complex mechanisms that can be symbolically represented as $X_1X_2Y : X_1X_1X_2X_2$ and $XY_1Y_2 : XX$ (where X_1 and X_2 represent genetically different X-chromosomes and Y_1 and Y_2 genetically different Y's). In the genus

Drosophila the great majority of the species have XY males, but in a few the males are XO and in *D. miranda* a centric fusion between the Y and one member of the third pair of autosomes has led to an X_1X_2Y condition in which the 'unfused' third chromosome has become the X_2. The Y of *D. miranda* includes not only the original Y-material but also the material of the 'fused' third chromosome, which has become broken up and re-distributed, no doubt by inversions and other types of structural rearrangements. In *D. americana* subspecies *americana* the males have become effectively XY_1Y_2 through a fusion between the X and one member of the fourth pair of autosomes. Similar transformations have occurred in grasshoppers, a group in which the primitive condition was undoubtedly an XO : XX one, which still persists in the overwhelming majority of the species. In about 25 genera, however, centric fusions between the X and acrocentric autosomes have led to XY mechanisms in which the unfused autosome has become a 'neo-Y'. In the Australian subfamily Morabinae this kind of transformation seems to have occurred independently at least seven times, and on five separate occasions in the phylogeny of this group further fusions between the neo-Y and another autosome created X_1X_2Y mechanisms.

The X_1X_2Y mechanism which is found in all members of about 15 genera of praying mantids has arisen in a different manner from those of the Morabinae, namely by a single translocation between a metacentric X and a metacentric autosome. This seems to be a unique instance where we can be confident that a group of several hundred species placed by taxonomists in a number of genera are a strictly monophyletic group, descended from a single species in which the X-autosome translocation became established.

In the plant kingdom polyploidy has played a major

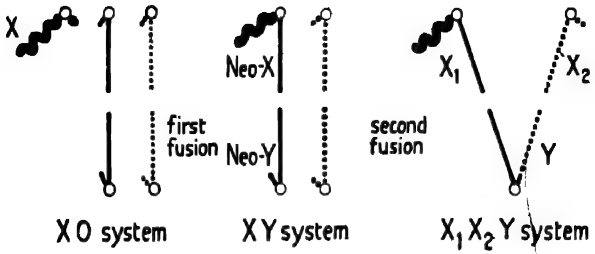


Fig. 24. Diagram showing how an XO sex chromosome mechanism may give rise, by a centric fusion, to an XY system and then, by a second fusion, to an X_1X_2Y one

This is how a number of species of grasshoppers have become X_1X_2Y in the male. Details of the centric fusion mechanism as in Fig. 16.

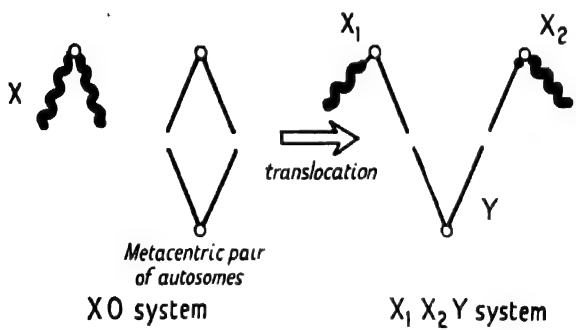


Fig. 25. Diagram showing how an XO system can give rise to an X_1X_2Y one by a single translocation if all the chromosomes are metacentric

This is how the X_1X_2Y mantids acquired their sex chromosome mechanism.

role in the evolution of many groups. In the algae evidence for polyploidy is rather scanty and in the fungi it may not exist naturally, but in the ferns, horsetails, clubmosses and psilotales it has occurred on a grand scale and some species have attained extraordinarily high chromosome numbers. However, the genus *Selaginella* invariably shows $n = 9$, all the very numerous species being diploids.

A few instances of tetraploidy have been reported in the gymnosperms, but polyploidy seems quite rare in this group, as it is also in many genera of woody angiosperms. In the angiosperms as a whole the proportion of polyploid species has been estimated at 30-35%. It seems to reach a maximum in the grasses, where some 75% of the species are polyploids.

A theoretical distinction exists between *autopolyploids*, in which all the chromosome sets are derived from the same species, and allopolyploids, which are derived by duplication of chromosome sets in interspecific hybrids. Starting with two diploid species whose genetic constitution may be symbolized as AA and BB we may have *autotriploids* AAA and BBB, *autotetraploids* AAAA and BBBB, diploid hybrids AB, allotetraploids AABB, etc. More complex polyploid types such as *allohexaploids* (AABBCC), *autoallohexaploids* (AAAABB) are also known. Almost all naturally occurring polyploid species in the plant kingdom are now believed to be allopolyploids, although it is a simple matter to produce autopolyploids by the use of colchicine and other drugs which induce chromosome doubling at mitosis. Some artificial autotetraploids are larger than the corresponding diploids, but this is not invariably so.

The evolutionary significance of allopolyploidy depends on the fact that it combines the genes of two or more species in one. Whereas most diploid hybrids

show some degree of sterility, due to incomplete pairing between the 'A' and 'B' chromosomes, an allotetraploid AABB may show high fertility because the two A sets will pair together and the B chromosomes will likewise. On the other hand, in certain allopolyploids pairing may occur between A and B chromosomes, in which case multivalents may be formed and some loss of fertility will result. Odd-numbered polyploids such as triploids, pentaploids, etc., will in any case have a very low fertility and can usually only establish themselves in nature if they rely on vegetative rather than sexual reproduction.

In such angiosperm families as the grasses repeated hybridization and chromosome doubling have produced what has been called a reticulate pattern of evolution. Nothing comparable to this is known in animals, although in certain weevils and earthworms that have almost or completely abandoned sexual reproduction for parthenogenesis high grades of polyploidy exist, and some of these forms (triploids, pentaploids, hexaploids, etc.) must have resulted from crossing, although not necessarily between members of different species.

Virtual proof of the origin of an allopolyploid from its presumed ancestors can only be obtained when those ancestors are not extinct, and when they can be crossed artificially, with subsequent doubling of the chromosome number. For example, it has proved possible to effectively re-synthesize the tetraploid species *Galeopsis tetrahit* (*G. pubescens* × *G. speciosa*) and *Nicotiana rustica* (*N. paniculata* × *N. undulata*) and the 12-ploid *Bromus arizonicus* (6*n B. haenkeanus* × 6*n B. trinii*) in this way. The case of the bread wheats (*Triticum vulgare* or *aestivum*) has been studied in great detail. These are allohexaploids, containing three diploid sets which have been called AA, BB and DD. The tetra-

ploid *durum* wheats have only AA and BB, while the diploid Einkorn wheats have only AA. The D genome is now known to have come from a grass *Aegilops squarrosa* probably after the cultivation of wheat by neolithic man, while the B genome is believed to have been derived at a much earlier date from *Ae. umbellulata* or *Ae. speltoides*.

Since most plant species are hermaphroditic there is no genetic mechanism for producing separate sexes to be upset by polyploidy. There can be no doubt that this accounts in part for the rarity of polyploidy in animals as compared with plants. It is not an entirely satisfactory explanation, however, since there exist hermaphroditic groups of animals, such as the Pulmonate Mollusca, in which polyploidy is unknown or confined to parthenogenetic species; and conversely there are dioecious plants such as the willows which exhibit polyploidy. In the former case the need for cross-fertilization is probably the main barrier to the establishment of polyploidy (two tetraploids would have to arise in the same population – otherwise mating of a tetraploid and a diploid would only produce sterile triploid offspring). In the latter case we are probably dealing either with sex-determining mechanisms that have arisen *since* the polyploid condition arose (*6n Rumex acetosella*) or with ones that depend on a single genetic locus rather than on a whole differential segment. In strictly bisexual groups of animals the only genuine polyploid species known seems to be the scale insect *Gossyparia spuria* in which sex-determination possibly does not depend on a genetic mechanism at all, the sex of the embryo being pre-determined by the nature of the egg cytoplasm, before fertilization.

We have already referred to the relative rarity of polyploidy among woody plants. Among the great genera of trees, the oaks (*Quercus*), pines (*Pinus*), figs

(*Ficus*) and eucalypts (*Eucalyptus*) show almost no polyploidy while a certain number of tetraploid species exist in *Populus*, *Acacia* and a larger number in *Salix*.

Polyploidy is most frequent among perennial herbs, especially in forms that are capable of vegetative as well as sexual reproduction. Among annuals it is relatively rare. Many polyploids undoubtedly suffer from reduced fertility at first and only gradually improve their sexual efficiency, as a result of natural selection. No doubt the ability to reproduce vegetatively has enabled many polyploid biotypes to pass through a period of reduced fertility which would otherwise have led to their extinction.

We have already referred (p. 146) to the existence of plant species which are permanently heterozygous for several or many translocations. Well-known examples are *Rhoeo discolor*, *Hypericum punctatum* and many species of the genus *Oenothera* (evening primroses). In these complex translocation heterozygotes rings of 6, 8, 10, 12, or 14 chromosomes are formed with great regularity at meiosis. The chromosomes are all metacentrics with limbs of approximately equal length, but in a species such as *Rhoeo discolor*, where all the chromosomes are included in the ring at meiosis, it is not possible to arrange the somatic chromosomes in pairs, since no one chromosome is completely homologous to any other. The chromosome set of a species such as *Oenothera muricata* (which forms a ring of 14) may be represented as follows:

ab C_1 *cd*	*lk* R_3 *mn*	*vu* C_6 *wx*
dc R_1 *ef*	*nm* C_4 *op*	*xw* R_6 *yz*
fe C_2 *gh*	*po* R_4 *qr*	*zy* C_7 $\alpha\beta$
hg R_2 *ij*	*rq* C_5 *st*	
ji C_3 *kl*	*ts* R_5 *uv*	$\beta\alpha$ R_7 *ba*

It will be seen that each chromosome consists of three segments, two terminal ones (represented by the small letters) and a median one, containing the centromere (symbolized by the capital C's and R's). The median segments are only imperfectly homologous; they may be regarded as *differential segments*, like those present in sex chromosomes. To a considerable extent, the individual must be haploid for genes present in the median segments.

At the first meiotic division, because of the regular zig-zag orientation of the chromosomes on the spindle all the C segments pass to one pole and all the R ones to the other. $C_1 \ldots C_7$ and $R_1 \ldots R_7$ are thus inherited as units, and they or the genes contained in them are referred to in this case as the *curvans* and *rigens* complexes. Similar complexes in other *Oenothera* species have received different names; for example in *Oe. lamarckiana*, studied by De Vries, which forms a ring of 12 and a single bivalent at meiosis, the complexes are called *velans* and *gaudens*.

Various mechanisms ensure that structural homozygotes are not produced. In *Oe. muricata* the *rigens* pollen grains are non-functional, only the *curvans* ones surviving; and on the female side almost all the embryo sacs receive a set of chromosomes carrying the *rigens* complex (the so-called 'Renner effect'). Thus the species is kept in a state of permanent heterozygosity. A different type of mechanism operates in *Oe. lamarckiana*, where the *velans* and *gaudens* complexes function in both pollen grains and embryo sacs, but the homozygous combinations are inviable as zygotes. The *muricata* mechanism may be referred to as the gametic type of balanced lethal system, the *lamarckiana* mechanism as the zygotic type. The species of *Oenothera* form a graded series from species like *Oe. hookeri* which are structurally homozygous and form seven bivalents to

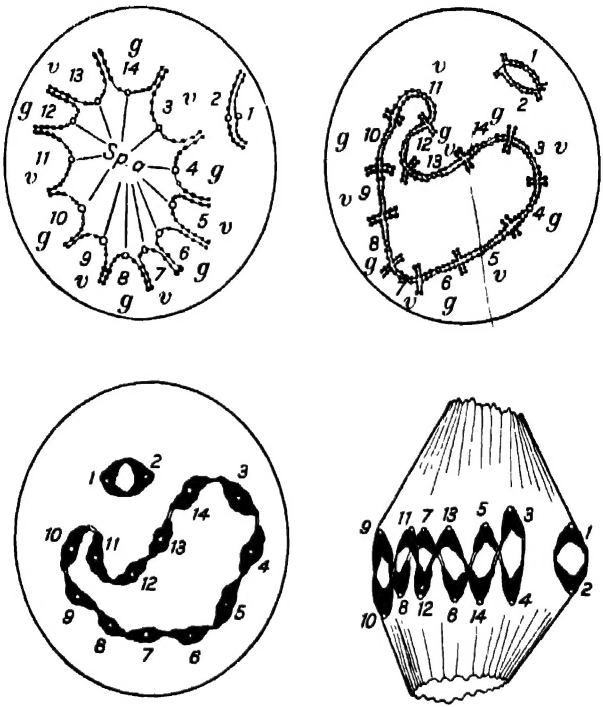


Fig. 26. Diagrams of the first meiotic division in Oenothera lamarckiana

The chromosomes are labelled 1–14. 1 and 2 form a bivalent, being homologous throughout. 3–14 form a ring of 12 chromosomes united by 12 chiasmata after diplotene. The middle segments containing the *velans* and *gaudens* complexes are labelled *v* and *g*. These segments contain the median centromeres. At first anaphase chromosomes 3, 5, 7, 9, 11 and 13 pass to one pole, while 4, 6, 8, 10, 12 and 14 pass to the other.

forms like *Oe. muricata* where all the chromosomes are included in the ring.

The so-called 'mutations' of De Vries, which caused much confusion in the early days of genetics and gave rise to the belief that new species arose by drastic *macromutations*, were of several sorts. One was simply an autotetraploid form of *lamarckiana*. Others were simply trisomics, produced with unusual frequency in *Oenothera* whenever malorientation of one chromosome in the ring leads to non-disjunction. Yet others were the result of occasional chiasmata between partially homologous median segments, leading in effect to crossovers between complexes and the formation of gametes, carrying part of one complex and part of another. Such 'mutations' are phenomena peculiar to the highly unusual *Oenothera* genetic system and are entirely different from true gene mutations.

The evidence from cytotaxonomy, from population genetics and from detailed studies of such phenomena as repeats, pseudoallelism and heterochromatin all indicates that the karyotypes of animal and plant species are highly organized systems rather than haphazard sequences of genes 'like beads on a string'. This is true, regardless of what view as to the actual molecular structure of the chromosome eventually prevails. The most remarkable fact is the great variety of different types of such systems known to exist, in view of the probably rather uniform chemical basis of heredity in higher organisms. Particularly striking are such alternatives as monocentric and polycentric chromosomes, euchromatin and heterochromatin, chiasmate versus non-chiasmate meiosis (male *Drosophila*), structurally homozygous species versus ones dependent on various forms of structural heterozygosity and the bizarre chromosome cycles of such insects as Sciara, gall midges and scales contrasted with more normal

chromosome cycles. At almost every stage in chromosomal evolution we meet with the phenomenon earlier called the 'principle of homologous change', but which may more appropriately be called *chromosomal orthoselection* – the repeated occurrence in evolutionary lineages of the same type of change in one chromosome after another. On the one hand, karyotypes must be mechanically harmonious and integrated systems – chromosome with chromosome and chromosomes with spindles and with all the dimensions of the cells. On the other hand, karyotypes must be genetically harmonious systems in which the advantages of co-adapted complexes of genes, of heterozygosity and homozygosity, of linkage and recombination, are combined and reconciled. When we consider the varied types of life cycles and the many different kinds of population structure met with in plants and animals the great variety of chromosomal mechanisms, although still very imperfectly understood as genetic systems, begins to take on meaning.